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## **Reproduction of Black Drum, *Pogonias cromis*, from the Chesapeake Bay Region.**

**Brian K. Wells<sup>1</sup>** and **Cynthia M. Jones**, Department of Biological Sciences, Old Dominion University, Norfolk, VA 23529.

### **ABSTRACT**

Ovaries of black drum, *Pogonias cromis*, collected from the Chesapeake Bay region in 1992, were used to describe reproductive strategy and fecundity. Histological examination showed that black drum spawn in the Chesapeake Bay region from April through early June. Distributions of oocyte diameter showed distinct oocyte-developmental groups indicating that Chesapeake Bay black drum are group-synchronous batch spawners. Female black drum are extremely fecund ranging from 414,000 to 3,736,000 hydrated oocytes (mean = 1,389,000) per batch with a spawning periodicity of 3.8 days. Estimates of spawning strategy, spawning periodicity, and batch fecundity for black drum from the Chesapeake Bay region were similar to reported estimates from the Gulf of Mexico.

### **INTRODUCTION**

In U.S. waters, where the primary range for black drum, *Pogonias cromis*, in the Northwest Atlantic is from the Delaware Bay south to Florida and through the Gulf of Mexico (Welsh and Breder, 1923; Hildebrand and Schroeder, 1928; Silverman, 1979), there are at least two known populations: one along the U.S. East Coast and one or more in the Gulf of Mexico (Gold *et al.* 1995; Jones and Wells 1998). Many essential elements of the reproductive life history are well understood for stocks in the Gulf of Mexico (Fitzhugh *et al.*, 1993; Nieland and Wilson, 1993; Saucier and Baltz, 1993; Fitzhugh and Beckman, 1987). Specifically, black drum from the Gulf of Mexico mature at five years, are group-synchronous spawners, with a batch fecundity of 1.4 to 1.6 million hydrated oocytes, and have a spawning periodicity of 3 - 4 days (Fitzhugh *et al.*, 1993; Nieland and Wilson, 1993).

In contrast to the Gulf of Mexico black drum, little is known about the reproductive biology of black drum along the U.S. East Coast. Studies of adult black drum in Eastern Florida and in the Chesapeake Bay have been limited to estimating the age at maturity and spawning season. Murphy and Taylor (1989) showed that the age at first maturity is five to six years and the spawning season along Northeast Florida is from January to May with peak spawning in March and April, while Alshuth and Gilmore (1995) found a more protracted spawning season from October to late March. In Virginia waters Bobko (1991) observed a spawning season of April through May, and noted that all fish were mature by age six. Although the length of the spawning season and age of maturity have been documented, spawning strategy has not been described nor batch fecundity estimated for black drum from along the U.S. East Coast.

Estimates of batch fecundity are essential to understanding life-time reproductive output. However, this information is difficult to obtain because an age-specific migration pattern exists for this population. Although black drum of all ages are present

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along Eastern Florida, with the majority of fish being very young, predominately old fish are found in the Chesapeake Bay and individuals younger than 10 are rare. This pattern is a result of size- and age-specific migrations, wherein larger, older fish expand their spawning range (Jones and Wells, 1998). Black drum in the Gulf of Mexico are not known to take the long-range spawning migrations that occur along the East Coast. Given this marked difference in behavior, other differences may also exist in reproduction between these populations.

We estimate the spawning strategy and batch fecundity of black drum within the Chesapeake Bay region, and compare our results to those for black drum of the Gulf Coast.

### MATERIALS AND METHODS

The persistence of black drum populations is determined by their lifetime reproductive output (Stearns, 1992). Reproductive output is, in turn, determined by survival, the average age of reproduction, and the annual, age-specific fecundity. The fundamental steps in estimating fecundity are to determine spawning type (total versus batch spawner) and annual fecundity (West, 1990). For a batch spawner, batch fecundity and spawning periodicity must also be estimated. Further, as reproductive output can differ between populations of the same species, estimates must be obtained specifically for spawning strategy, annual fecundity, and average reproductive lifespan from each separate population (West, 1990). For example, populations of American shad (*Alosa sapidissima*) exhibit profoundly different reproductive characteristics along the U.S. East Coast: they are semelparous in the south and iteroparous in the north (Scott and Scott, 1988). Although such dramatic differences are rare, they underscore the importance of determining the reproductive patterns of a fish species throughout its range.

We collected ovarian samples from freshly caught black drum from early April through early June 1992 from the Virginia commercial and recreational fisheries on the bayside and seaside of the eastern shore of the Chesapeake Bay. Additional ovarian samples were obtained opportunistically from the recreational fishery in the Maryland portion of the Chesapeake Bay during 9-16 June 1992.

Commercially-caught fish were taken with anchored, monofilament gill nets of 33 cm bar mesh on the seaside of the eastern shore of Virginia in channels between barrier islands and on the bayside in waters just off Cape Charles. Both locations are sites of black drum spawning (Daniel, 1995). Fishers set their nets in early evening and fish overnight during expected periods of peak spawning (Mok and Gilmore, 1983; Fitzhugh *et al.*, 1993; Nieland and Wilson, 1993; Saucier and Baltz, 1993; Daniel, 1995) and also use multiple nets that they simultaneously drift over drumming aggregations of black drum. From the recreational fishery, we sampled hook and line caught fish, throughout the day from locations mostly off Cape Charles where 90% of the recreational catches are typically landed (Jones and Wells, 1998).

Spawning season for males was described by examining gonadosomatic indices (total gonad weight  $\times$  100/ total body weight). We determined spawning season and fecundity for females by directly examining their ovaries. Unfixed ovarian sections were sieved to release oocytes from tissue following methods presented in Lowerre-Barbieri and Barbieri (1993). Once oocytes were freed from sections, they were preserved in 5% formalin. Smaller ovarian samples (1-2 cm<sup>3</sup>) were preserved in 10% buffered formalin for histological examination. All ovarian samples were taken from



the medial region of the fresh ovary and included a cross section of tissue from tunica to lumen. The medial region was sampled as our preliminary studies, and those of Nieland and Wilson (1993) and Fitzhugh *et al.* (1993), showed no statistical differences in development or estimates of fecundity among ovarian lobes or among ovarian regions for black drum.

Maturity stages were determined by direct histological examination of fixed ovary sections. Preserved ovarian samples were dehydrated, embedded in paraffin, sectioned to 5 microns, and stained with hematoxylin and eosin Y following Luna (1968). These sections, were used to identify four developmental stages following descriptions in Overstreet (1983), Murphy and Taylor (1989), and Morrison (1990) based on the most advanced oocyte stage and ovarian condition (stages of interest included yolked oocytes, hydrated oocytes, redeveloping ovaries showing postovulatory follicles, and spent).

Spawning strategy was determined from size-frequency distributions of oocytes and histological examination (Clark, 1934; Wallace and Selman, 1981; West, 1990). We placed aliquots of suspended oocytes in a gridded settling dish (0.5 cm<sup>2</sup> grids) and measured oocyte diameters from randomly selected grids until a minimum of 1000 oocytes had been measured from each ovary. We compared resulting distributions of whole-oocyte diameters to those of oocytes in different developmental stages that we observed in histological sections. This allowed us to associate size modes to oocyte maturation stage (Clark, 1934; Wallace and Selman, 1981; West, 1990).

Batch fecundity was estimated gravimetrically from hydrated oocytes (Hunter *et al.*, 1985). We used only fish that had no postovulatory follicles, which would have indicated a loss of oocytes before the gonads were excised, and estimated a mean fecundity from two subsamples from each fish (one fish had a single subsample). The number of hydrated oocytes estimated for each sample of known weight (0.2 - 3.5g, mean = 1.4g,  $N = 13$ ) was expanded to the entire ovary.

Spawning periodicity was estimated from the fraction of fish with postovulatory follicles to all fish with healthy vitellogenic oocytes (Hunter and Goldberg, 1980). We assumed that all of the postovulatory follicles were approximately the same age based on estimates that postovulatory follicle duration is limited to 24 to 48 hours following ovulation (Fitzhugh *et al.* 1993).

## RESULTS

In total, we obtained only 351 black drum despite our almost daily sampling over two and one-half months when the fish were most abundant. Of these, 71 were females and only 54 were considered fresh enough for ovarian examination. Of the 54 fresh ovarian samples, we obtained 37 from the commercial fishery and 17 from the recreational fishery. Also, because we were limited to using only hydrated females caught on the day of sampling, our sample size for batch fecundity estimates was small ( $N = 7$ ). Early in the season, relatively few black drum were captured either by the commercial or recreational fishery. Large catches occurred in the last three weeks of May but were predominately males. Complete reproductive data was taken from only 83 males to accommodate fishers who dressed their catch while we sampled.

Histological examination showed that the black drum spawn in the Chesapeake Bay region from April through early June (Fig. 1). Fish entering the Chesapeake Bay region in April already had yolked oocytes (Fig. 1) and some were redeveloping, indicated by

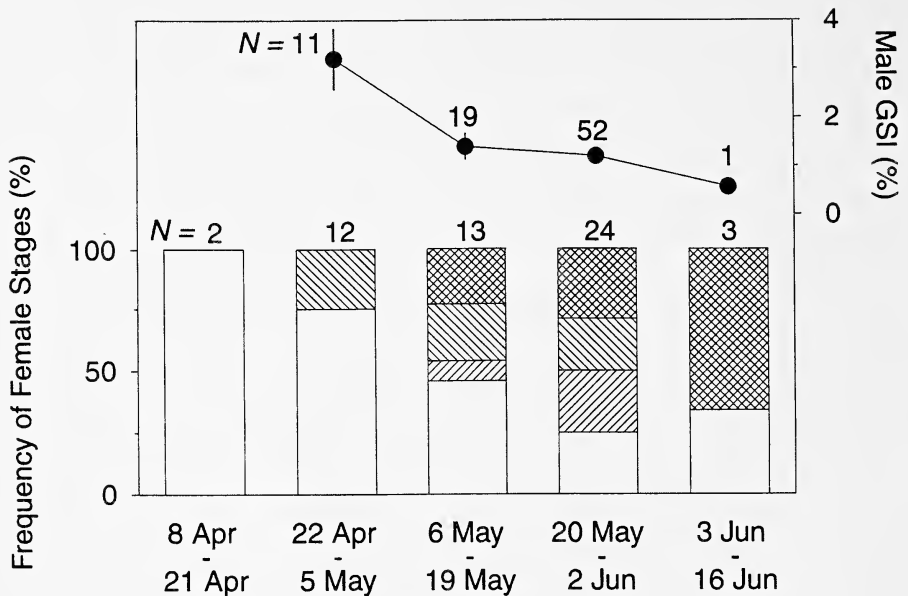


FIGURE 1. Proportions of maturity stages identified in the Chesapeake Bay region sampled 8 April - 16 June 1992. Maturity stages represented are yolked (clear), hydrated (forward diagonal), redeveloping (back diagonal), and spent (cross hatch). Virginia waters were sampled daily from 8 April - 2 June 1992 and Maryland waters were sampled 9 - 16 June 1992. Bars represent the sampled ovaries grouped biweekly. Also shown are the average gonadosomatic indices (GSI, 95% confidence intervals shown by bars) for males during the same time periods represented for females. Notice that decline in GSI values occurs as the proportion of spent ovaries increases supporting the argument that the spawning season is ending by early or mid June. Sample sizes ( $N$ ) are shown above each bar and average GSI.

the presence of fresh postovulatory follicles. By early June the proportion of spent ovaries increased and that of hydrated, developing, and redeveloping ovaries decreased indicating that the black drum spawning season in the Chesapeake Bay region was April through early June. Male gonadosomatic indices decreased steadily from ~4% in April to ~0.5% by early June ( $N = 83$ , Fig. 1). The end date of the spawning season could not be confirmed because fishing stopped in early June, although we observed few maturing fish by this time.

Distributions of oocyte diameter ( $N = 32$ ) showed distinct oocyte-developmental groups indicating that Chesapeake Bay black drum are group-synchronous batch spawners as defined by Wallace and Selman (1981). As an example, Figure 2 illustrates a series of size-frequency plots representing different stages of a spawning cycle. The distributions consistently showed these distinct peaks for all females. We identified postovulatory follicles in 11 ovaries out of 42 with healthy vitellogenic oocytes from direct histological examination, from this we estimated spawning periodicity was 3.8 days.

Black drum in the Chesapeake Bay region are extremely fecund ranging from 414,000 to 3,736,000 hydrated oocytes (mean = 1,389,000,  $N = 7$ ) (Table 1). Using the estimated spawning periodicity of 3.8 days and a spawning season of 45 days (mid

TABLE 1. Estimates of batch fecundity and standard errors (SE) obtained from hydrated ovaries. The gravimetric method was used to calculate the mean number of hydrated oocytes from two subsamples from each ovary.

Capture date	Total length (cm)	Total weight (kg)	Age (yr)	Batch fecundity (SE)
18 May	98	15.88	18	897,43 (66,847)
20 May	109	18.14	27	1,423,888 (155,985)
21 May	104	17.69	31	414,307 (104,103)
23 May	111	---	23	459,407 (27,644)
23 May	120	---	30	2,190,169 (one subsample)
23 May	109	---	22	601,198 (75,092)
30 May	109	---	29	3,736,196 (143,583)
Mean	108	17.24	25	1,388,943

April to early June) the total fecundity of an individual black drum in the Chesapeake Bay region could be 16.4 million hydrated oocytes. Although fecundity is often related to size, regressions of length, total weight, and age on batch fecundity estimates were not significant ( $P > 0.05$ ).

### DISCUSSION

Until now, there has been a lack of fundamental knowledge of reproduction in black drum, a requisite to manage this population along its entire U.S. range. Although Fitzhugh *et al.* (1993) and Nieland and Wilson (1993) have determined spawning strategy, spawning periodicity, and have estimated the batch fecundity of black drum in the Gulf of Mexico, none of this was known for black drum from the East Coast population. This first examination indicates that reproductive strategy and spawning periodicity of black drum of the Chesapeake Bay region is similar to that from other areas of the U.S. geographic range of the species. Black drum from the East Coast are group-synchronous spawners similar to those in the Gulf of Mexico as reported by Fitzhugh *et al.* (1993) and Nieland and Wilson (1993). Individuals may spawn starting as early as October (Alshuth and Gilmore, 1992) or January (Murphy and Taylor, 1989) and until at least early June in the Chesapeake and Delaware Bays (these results; Thomas and Smith, 1973). This long spawning season on the East Coast is similar to Gulf of Mexico black drum where their spawning season is approximately five months (Fitzhugh *et al.*, 1993; Nieland and Wilson, 1993). Our estimate of spawning periodicity (3.8 days) is also similar to estimates of 3-4 days for Gulf of Mexico black drum (Fitzhugh *et al.*, 1993; Nieland and Wilson, 1993).

Batch fecundity of Chesapeake Bay black drum (1.4 million hydrated oocytes) is similar to fecundities estimated for the Gulf of Mexico reported by Fitzhugh *et al.* (1993) and Nieland and Wilson (1993). Typically, larger, older fish of a species are more fecund (West, 1990). Chesapeake Bay black drum are older and larger on average (109.5 cm) than those in the Gulf of Mexico (76 cm; Fitzhugh *et al.*, 1993); however, they are not more fecund (1.4 to 1.6 million hydrated oocytes; Fitzhugh *et al.*, 1993; Nieland and Wilson, 1993). We suggest three hypotheses to account for this. First, older fish from the Chesapeake Bay region undertake long-range spawning migrations that Gulf of Mexico fish do not. Energy spent in undergoing this migration may reduce their reproductive output. Second, because black drum reach asymptotic growth early (10 - 20 years) relative to their life-span of sixty years, the size range of mature fish is

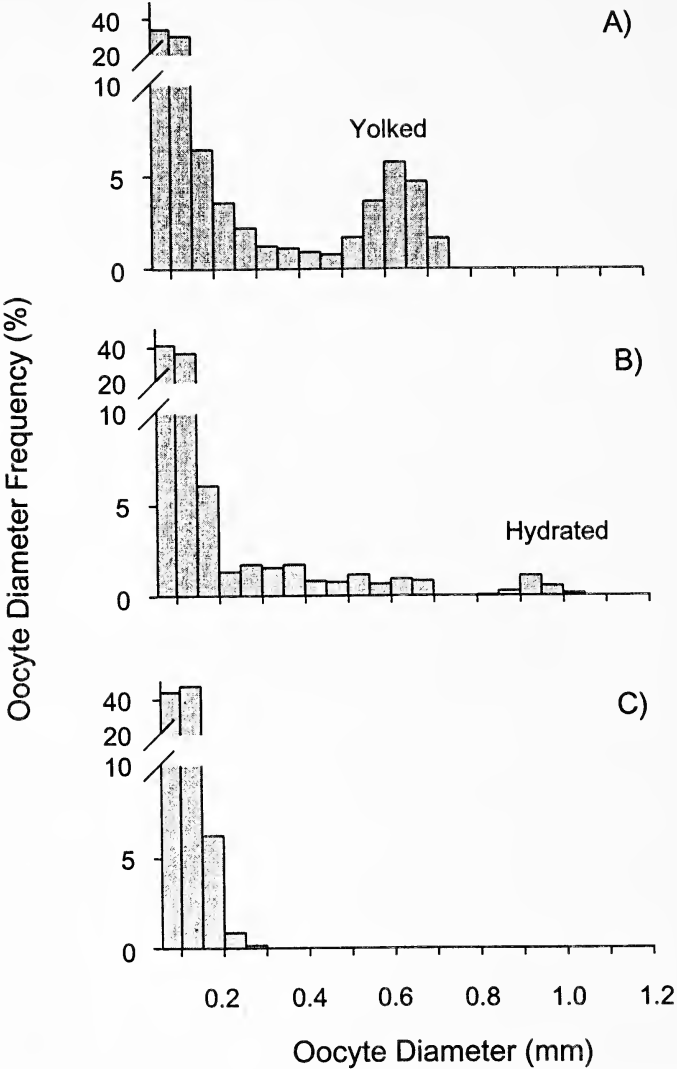


FIGURE 2. Distributions of whole-oocyte diameter for the four stages of maturity identified in adult black drum within the Chesapeake Bay region. Maturity stages represented are a) yolked or redeveloping, b) hydrated, and c) spent. Notice the stages are defined by the most advanced stage of oocyte development and the occurrence of postovulatory follicles as identified using histology. Also note that within an ovary classified as yolked, hydrated, or redeveloping are multiple stages of oocyte development indicating continued spawning once the most advanced stage oocytes hydrate and release. Further, as the oocyte diameter modes are distinct black drum is determined to be a group-synchronous spawner releasing clutches of eggs periodically.

limited so that relationships of fecundity to size and age are poor (Nieland and Wilson, 1993) or insignificant (Fitzhugh *et al.*, 1993). Finally, it may be that East Coast black drum are less fecund at reproductive age.

From our estimates of mean batch fecundity and spawning periodicity, East Coast black drum are capable of producing approximately 56 to 98 million hydrated oocytes in 40 to 70 batches per female during a five to nine month spawning season. As black

drum mature between five and six years, and have a mean maximum age of almost 60 years, these fish have the potential for over fifty years of reproduction. This is similar to the reproductive effort of fish with three times the natural mortality of black drum (Gunderson and Dygert, 1988). Yet, the prodigious reproductive potential of black drum has not resulted in large populations.

We propose that this reproductive strategy is an adaptation to great environmental variability along a geographic gradient characterized by large intra- and interannual temperature and salinity variation. The Chesapeake Bay has sporadic recruitment success as indicated by occasional large numbers of young-of-the-year black drum exiting the bay during the fall. Joseph *et al.* (1964) suggested aperiodic recruitment success is a result of intolerable abiotic factors because the Chesapeake Bay is at the northern extension of the fish's spawning range. For example, winter temperatures in the Chesapeake and Delaware Bays drop below 4°C, the lower thermal lethal limit for other sciaenids (Lankford and Targett, 2001). Moreover, Cowan *et al.* (1992) and Daniel (1995) concluded that poor and episodic recruitment from the Chesapeake Bay is due to a short spawning season which overlaps times when the density of jellyfish predators is great. Such high egg and early-life mortalities are common for a highly fecund fish that has adapted to environmental variability. It follows that during years of favorable environmental conditions and low predator abundances, theoretically, recruitment from the Chesapeake Bay will be high. Therefore, we hypothesize black drum have a reproductive strategy adapted to biotic and abiotic variability such that the contribution of recruits from the Chesapeake Bay to the whole East Coast population will be variable.

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## **Autotrophic Picoplankton: Their Presence and Significance In Marine and Freshwater Ecosystems<sup>1</sup>**

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During the first half of the 20<sup>th</sup> century, scientists collecting plankton specimens would use nets having different sized apertures to selectively obtain organisms within various plankton categories. As these net apertures were reduced in size, it was realized that there were numerous microscopic cells capable of passing through the smallest openings of these nets (Lohmann, 1911). The presence of these very small cells was later reported at numerous freshwater sites (Rodhe, 1955; Bailey-Watts et al., 1968; Pennak, 1968; Votintsev et al., 1972; Pearl, 1977) and marine locations (Van Baalin, 1962; Saijo, 1964; Saijo and Takesue, 1965; Reynolds, 1973; Banse, 1974; Berman, 1975; etc.). In this early literature, various terms were used to describe these cells (e.g. ultraplankton, olive green cells,  $\mu$ -algae, nanoplankton, etc.), but it wasn't until Sieburth et al. (1978) established a plankton reference classification system based on size, that the term picoplankton began to be used collectively for these microscopic cells. The standard definition of picoplankton refers to cells within the size range of 0.2 to 2.0 microns. This term has since been generally accepted as the category to assign plankton cells that occur singly or within colonies that are within this size range. However, one of the initial concerns in algal studies was the inability to distinguish many of the bacteria, cyanobacteria, and eukaryotes in this category with similar characteristics, and to specifically identify the heterotrophs from autotrophs when limited to standard light microscopy protocols.

A major contribution regarding the identification of picoplankton components came from Johnson and Sieburth (1979) and Waterbury et al. (1979). Using epifluorescence microscopy, they identified abundant and widely distributed phycoerythrin containing cyanobacteria as the common component of the picoplankton community in the world oceans. These were chroococcalean taxa which were identified as *Synechococcus* and occurring at concentrations up to  $10^4$  cells/mL. They recognized the high abundance and broad distribution of these cells in marine waters and suggested their likely importance as a food source for microzooplankton. This significance was also indicated by productivity studies in the tropical north Atlantic equatorial current by Gieskes et al. (1979). They reported 20-30% of the productivity and 43-53% of the chlorophyll *a* measured was passing through a  $1.0\mu$  filter, which indicated a major portion of the total productivity from this region was derived from cells  $<1.0\mu$  in size. Other investigations followed that supported the wide spread presence of picoplankton in freshwater lakes (Chang, 1980; Cronberg and Weibull, 1981; Craig, 1984). Similar picoplankton studies using epifluorescence procedures with fluorochrome dyes were able to further distinguish the heterotrophic bacteria from the prokaryotes and eu-

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1 Invited lecture: Autotrophic Picoplankton Workshop, 52<sup>nd</sup> Congress of the Polish Botanical Society, Adam Mickiewicz University, Poznan, Poland, September 2001.

karyotes within water samples (Porter and Feig, 1980; Davis and Sieburth, 1982; Caron, 1983), with the term autotrophic picoplankton, or picophytoplankton commonly used for the autotrophic cells (Fogg, 1986). Review articles on marine and freshwater picoplankton include those by Fogg (1986), Joint (1986), Stockner and Anita (1986), Stockner (1988; 1991), and Stockner et al. (2000).

Although the term picoplankton is applied to cells 0.2 to 2.0  $\mu$  in size, there is the general acceptance by investigators to include cells having a larger size range, but still capable of passing through a 2.0  $\mu$  pore filter. A broader size range of cells, for instance  $<3\mu$  in size, was generally considered a more natural upper size limit to characterize these cells, many of which were referred to as unidentified cells (e.g. cells  $<3\mu$ ) when using standard Utermöhl protocols (Saijo, 1964; Reynolds, 1973, Munawar and Fahnenstiel, 1982; Marshall, 1982; 1983). The majority of these cells are considered somewhat pliable, and may be ellipsoid, spherical, or rod shaped, with cell diameters  $<2\mu$ , but with their length often longer, and yet still be capable of passing through a 2.0  $\mu$  filter. In their measurements of 14 chroococcales picoplankton isolates, Smarda and Smajs (1999) noted variability in their dimensions and recorded the average cell length was from 0.8 to 2.9  $\mu$ , but having widths between 0.6 and 2.0  $\mu$ . However, as more taxa within this category were discovered, it became evident that there were large concentrations of cells at the lower ranges of this size category. Joint (1986) would favor the picoplankton definition to be expanded to identify more specifically cell groups within this size range. For instance to recognize those cells that can pass through a 1.0  $\mu$  pore size filter from larger cells, so the significance of these much smaller cells can be established. A further breakdown of this size fraction would provide more specific information regarding their contribution to productivity and prey relationships. Viewpoints of other investigators consider in addition to size, that there are other criteria that may be applied to taxa described as picoplankton. For instance, there are numerous and common freshwater colonial cyanobacteria with cells  $<2.0\mu$  in size (e.g. *Aphanothece*, *Aphanocapsa*, *Merismopedia*, etc.). Komarek (1996) also indicates the term should be limited to those unicellular taxa that appear singly in the water column, rather than as colonial clusters. This is in consideration of the different ecological relationships and descriptive taxonomy that may be associated with these different colonial species. In addition, the gelatin matrix within which these cells are found may interfere with their passage through the smaller apertures of the filters used, producing an incomplete representation of this category. Komarek (1996) recommends species identification should go beyond morphology and genetic makeup, and include specific ecophysiological relationships unique to the taxon. Many investigators will combine standard Utermöhl procedures in determining the composition and abundance of the colonial picoplankton, and the epifluorescence protocols for recording the single cell taxa among the picoplankton (Marshall and Nesius, 1993; 1996; Marshall and Burchardt, 1998; among others).

#### COMPOSITION

Following the publications of Johnson and Sieburth (1979) and Waterbury et al. (1979), it was common practice in freshwater and marine studies to identify the picocyanobacteria as *Synechococcus*. However, many of these cells exhibited different morphological features and were collected from extremely diverse habitats. Questions regarding the possible presence of other taxa within this assemblage were

aided during the past two decades by the application of autofluorescence, electron microscopy, and genetic analysis. These approaches have been essential in the identification of various strains and species within the *Synechococcus* complex, in addition to other picoplankton species (Rippka and Cohen-Bazere, 1983; Waterbury and Rippka, 1989; Leppard et al., 1989; Corpe and Jensen, 1992; Hall, 1991; Olson et al., 1993; Komarek, 1994, 1996, 1999; Komarek and Cepak, 1998; etc.). In general, the most common and abundant taxa within the autotrophic picoplankton category are cyanobacteria (e.g. in the genera *Synechococcus*, *Cyanobium*, *Cyanothece*). If the colonial cyanobacteria are included in the category, species from the genera *Aphanocapsa*, *Aphanothece*, *Chroococcus*, *Merismopedia*, etc., would be added. In addition, among these taxa are differences in the type and amount of pigments they contain. For instance, phycoerythrin-rich prokaryotes are in high concentrations within oceanic regions (Mousseau et al. (1996), whereas, phycocyanin-rich taxa are more common in estuaries (Ray et al., 1989; Lacouture et al., 1990), and when passing through salt water-freshwater transition zones (Bertrand and Vincent, 1994). In these areas, the ratio between phycoerythrin and phycocyanin rapidly decreases.

Another major phylogenetic category is represented by the eukaryotes. These include the chlorophytes (e.g. *Chlorella* spp., *Micromonas pusilla*) and other major phytogenetic groups (e.g. chrysophytes, haptophytes, prasinophytes, etc.) that collectively contribute to the picoplankton biomass and productivity (Thomsen, 1986; Stockner, 1988; Hargraves et al., 1989). Also reported from the world oceans are the prochlorophytes (Chisholm et al., 1988), with *Prochlorococcus marinus* a common species (Chisholm et al., 1992). These are among the smallest photosynthetic organisms known. This is a unique prokaryotic group with species that contain divinyl derivatives of chlorophylls *a* and *b*, *a*-carotene and zeaxanthin, with a thylakoid feature of having them closely arranged in the cell (similar to the original Type II cell described by Johnson and Sieburth, 1979, Alberte et al., 1984; and others). These cells are 0.6-0.8 X 1.2-1.6  $\mu$  in size and have been recorded in coastal and oceanic waters (Chisholm et al., 1988, 1992; Shimada et al., 1995), and appear to be ubiquitous within the photic zone of tropical and sub-tropical oceans. Jochem (1995) also reports prochlorophytes as common in the lower extent of the euphotic zone and as dominant flora below the chlorophyll maximum regions, with *Synechococcus* abundant from sub-surface waters to the bottom of the euphotic zone.

Since the picoplankton cells lack many of the morphological features necessary for distinguishing between species, a more exact genetically-based approach is to use polymerase chain reactions (PCR) (Krienitz et al., 1999; among others). This method provides for the amplification and identification of specific gene sequences that are used to identify these species and to resolve questions regarding whether taxa with similar phenotypic or morphological characteristics represent one or more species, or strains of a species. Through this type of analysis additional strains of common prokaryote species, plus an array of new eukaryote species, including haptophytes, chlorophytes, prasinophytes, and others have been identified (Moon-van der Staay et al., 2000, 2001; Lopez-Garcia et al., 2001). Examples from fresh water habitats include: *Nannochloropsis limnetica* (eustigmatophyte) and *Pseudodictyosphaerium jurisii* (chlorophyte) by Krienitz et al. (1999, 2000); and from marine waters *Chrysochromulina fragaria* (prymnesiophyte) by Eikrem and Edvardsen (1999), plus a new class and species designation of *Bolidomonas* spp. (Polidophyceae) by Guillou

et al. (1999), and *Pelagomonas calceolata* (Pelagophyceae) by Anderson et al. (1993). A listing of many common picocyanobacteria is given by Stockner et al. (2000).

#### ABUNDANCE WITHIN COASTAL AND DEEP OCEAN REGIONS

Numerous marine studies have indicated a positive correlation of picoplankton abundance to temperature. These include those by Murphy and Haugen (1985), El Hag and Fogg (1986), Waterbury et al. (1986), Jochem (1988), Iriarte and Purdie (1994), etc., plus in estuary studies by Malone et al. (1991), Marshall and Nesius (1993, 1996), Marshall (1995), and Davis et al. (1997). In California coastal areas, Krempin and Sullivan (1981) found the picoplankton abundance lowest in winter, then gradually rising through spring to peak in fall ( $10^7$  cells/mL). Davis et al. (1985) found similar findings in Narragansett Bay with highest concentrations from spring through fall. In the north Atlantic, picoplankton concentrations decrease moving from the coastal waters to less nutrient and more stable regions seaward at the surface and within the euphotic zone (Platt et al., 1983; Murphy and Haugen, 1985; Glover et al., 1985b). In a transect between the Gulf of Maine and the Sargasso Sea, Glover et al. (1985a) found cyanobacteria the most abundant picoplankton in the eutrophic region over the continental shelf (88-98%). Concentrations of the (phycoerythrin-rich) cells ranged from  $1.3 \times 10^3$  to  $1.5 \times 10^5$  cell/mL, with the eukaryotes ranging from  $0.3 \times 10^5$  to  $4.4 \times 10^4$  cell/mL. The cyanobacteria abundance in comparison to other categories within the picoplankton, was greatest in the least productive regions (representing 91% of the autotrophic picoplankton), and lowest (65.7%) in the well mixed and more productive sites where they co-existed with other abundant taxa (eukaryotes). However, seaward into the open ocean the eukaryotes became more dominant in the lower regions of the photic zone. At one station, with light transmission levels of 4%, 1%, and 0.5%, the eukaryotes represented 73%, 78%, and 70% of the picoplankton respectively. The autotrophic picoplankton represented 70-97% of the phytoplankton chlorophyll and 73-78% of the autotrophic picoplankton at the deep chlorophyll maximum, showing an increased ratio between eukaryotes and picocyanobacteria with depth.

A similar pattern was reported by Murphy and Haugen (1985) with the picoplankton more abundant at coastal sites in the North Atlantic ( $10^7$  to  $10^8$  cells/mL), with decreasing abundance seaward ( $10^6$  to  $10^7$  cells/mL). In the surface waters, the cyanobacteria were more abundant than the eukaryotes, but the eukaryotes were often in greater concentrations with increased depth, being dominant at the lower ranges of the photic zone (150-200m). These eukaryotes and the prokaryotes were in high concentrations at and below the compensation depth. Often high concentrations of eukaryotes (up to 50% of the picoplankton population) have been reported within the oceans (e.g. Hall and Vincent, 1990). Also, Zubkov et al. (2000) recorded *Prochlorococcus* at concentrations of  $10^6$  cells/mL dominating the other two categories in the oligotrophic gyre regions of the Atlantic Ocean, but within the more productive waters of the equatorial region, both the eukaryotes and pico-cyanobacteria were more abundant. Summer prokaryote concentrations in the Baltic were reported by Sondergaard et al. (1991) to be at  $10^6$  to  $10^8$  cell/mL. Within a polynesian atoll, Charpy and Blanchot (1998) recorded the picoplankton showed a diurnal variation in their size, being smaller before sunrise, and becoming larger by the afternoon hours. Within these waters the average size for the pico-eukaryotes, *Prochlorococcus*, and *Synechococcus*

were 3.11, 0.89, and 0.62  $\mu$  respectively. Dimensions in the literature vary for these categories. However, common ranges reported for these groups have included up to 3.26  $\mu$  for the pico-eukaryotes, 0.55 to 1.0  $\mu$  for *Prochlorococcus*, and 0.6 to 2.2  $\mu$  for *Synechococcus* spp.

Although picoplankton cell abundance typically decreases with depth at near shore and ocean sites, a sub-surface zone of increased abundance is commonly found. Krempin and Sullivan (1981) recorded this at 30 m off the California coast, and high cell concentrations were also associated with the chlorophyll maximum layer in the north Pacific at 60 m depth (Takahashi and Hori, 1984). At this depth, more than 70% of the chlorophyll was represented by autotrophic picoplankton ( $<3 \mu$ ), with the two dominant taxa being a *Chlorella*-like eukaryote species (1.2 - 1.5  $\mu$ ) and a prokaryote. (0.5 - 2.0  $\mu$ ). Other picoplankters included prasinophyte and haptophyte taxa, and non-thecate dinoflagellates. Within the Kiel Bight region, Jochem (1988) reported peak cyanobacteria picoplankton abundance in summer and increasing more in the eutrophic areas ( $10^8$  cells/mL), than in less eutrophic regions. During summer months, 8-52% of the total phytoplankton carbon, and up to 97% of the autotrophic picoplankton carbon came from the picocyanobacteria. High cell concentrations occurred during the late summer or early fall (e.g. eukaryotes abundance at  $10^6$  cells/mL, Hargraves et al., 1989). Deeper presence of picoplankton was reported by Li and Wood (1988) in the central north Atlantic to depths of 220 m, in addition to high concentrations ( $>90\%$ ) of eukaryotes. They also report a sub-surface maximum at 70 m, then a decrease in cell abundance with increased depth. The contribution of the autotrophic picoplankton to total carbon production in the oligotrophic and less disturbed regions of the ocean is estimated as 50-90%, in contrast to the meso-eutrophic coastal regions where it much lower, 2-25% (Stockner and Antia, 1986). Due to the lesser abundance of the larger phytoplankters in these open oceanic areas of low nutrient input, and where there occurs periods of extended stratifications and reduced nutrient entry, the percent contribution of picoplankton as a primary producer becomes greater.

As mentioned previously, there is a difference in the ratio of phycocyanin and phycoerythrin pigments in picocyanobacteria cells found in the estuaries and pelagic regions. Phycoerythrin-enriched cells are more dominant in the oceanic and coastal regions (Waterbury et al., 1979; Johnson and Sieburth, 1979; Takahashi and Hori, 1984; Murphy and Haugen, 1985), with the phycocyanin-enriched cells more dominant in the less saline regions of tidal rivers and bays (Ray et al., 1989; Lacouture et al., 1990; Bertrand and Vincent, 1994). In the York River estuary (Virginia), Ray et al. (1989) determined the autotrophic picoplankton (0.2-3.0  $\mu$ ) represented 7% of the phytoplankton biomass and 9% of the primary production. The picoplankton was dominated by phycocyanin-enriched cyanobacteria, which were 8 times greater in abundance than the phycoerythrin-enriched cyanobacteria cells. The combined concentrations of these cells were  $10^5$  cells/mL and they represented 51% of the picoplankton biomass. The remaining biomass was by flagellates, diatoms, etc. This study also recognized increased abundance of the picoplankton coincided with the spring neap tide, a period where the water column is more stable and stratified. In the Patuxent River, a Chesapeake Bay tributary, the cyanobacteria were the major component of the autotrophic picoplankton, having a seasonal abundance maximum after the spring diatom bloom (Lacouture et al., 1990). They accounted for up to 50% of the summer

phytoplankton productivity during this period. Affronti and Marshall (1993) conducted diel studies during August and January at the Chesapeake Bay entrance. The phycocyanin enriched *Synechococcus* was the dominant prokaryote in August and the highest concentrations occurred during ebb tide. The average August pico-abundance was  $8.84 \times 10^5$  cells/mL and  $1.43 \times 10^5$  cells/mL, above and below the pycnocline respectively. There were greater concentrations of the phycocyanin enriched cyanobacteria above the pycnocline, in contrast to higher concentrations of the phycoerythrin enriched cells below the pycnocline in the deeper more saline waters entering the Bay. In January, the mean picoplankton abundance was  $3.65 \times 10^4$  cells/mL and  $4.66 \times 10^4$  cells/mL, above and below the pycnocline respectively. At this time the phycoerythrin enriched *Synechococcus* cells were dominant throughout the water column. In a 41 month study of picoplankton concentrations at 7 stations in Chesapeake Bay, Marshall (1995) reported their cell abundance was closely associated to temperature, forming a single annual maximum that peaked in July or August. The monthly means ranged from a  $9.6 \times 10^3$  (February) to  $907.0 \times 10^3$  (August) cell/mL. The sub-pycnocline concentrations were less than those above the pycnocline between May and November, but were slightly higher from December through May. In several tidal tributaries to the Chesapeake Bay, the autotrophic picoplankton produced typical summer maxima and winter population lows where their monthly concentrations ranged from  $3\text{--}5 \times 10^3$  to  $10^5$  cells/mL (Lacouture et al., 1990; Davis et al., 1997; Marshall and Burchardt, 1998). Campbell et al. (1983) in comparisons between *Synechococcus* and *Synechocystis* presence, found phycocyanin rich *Synechococcus* cells a minor component in Great Bay, N.Y. during spring and summer, being dominated by phycoerythrin rich *Synechococcus* cells from summer through late autumn.

Thus, picoplankton composition decreases in abundance, moving from off shore and continental shelf regions seaward (Murphy and Haugen, 1985; Stockner and Anita, 1986). Although they are in lesser abundance in these open oceanic regions in comparison to their concentrations in coastal waters, they contribute a greater percentage of the total algal productivity in comparison to those algal cells larger than  $2\text{--}3 \mu$ , and represent a significant contributor to the total productivity in these less nutrient enriched regions (Stockner and Anita, 1986). Due to their ability to better utilize low intensities of light and existing nutrients in comparison to larger size phytoplankters, they are abundant throughout the photic zone, with regions of high concentrations at the surface, and in a sub-surface maximum, plus being abundant at the lower range of the euphotic zone. The picocyanobacteria generally are found in greater concentrations than the eukaryote species, especially in oligotrophic regions. Seasonal periods associated with peak abundance of picoplankton in temperate waters occur during summer and/or early fall, whereas, in tropical waters the concentrations are more constant. The typical concentrations in oceanic waters are approximately  $10^3$ ,  $10^4$ , and  $10^5$  cells/mL for eukaryotes, cyanobacteria, and prochlorophytes respectively (Fogg, 1986; Stockner, 1988; Caron et al., 1985), and represent a common range of 2-25% and 50-80% of the total oceanic primary production in eutrophic coastal waters and oligotrophic regions respectively.



## ABUNDANCE WITHIN FRESHWATER LAKES

The autotrophic picoplankton have long been recognized as an abundant and ubiquitous component of freshwater lakes (Hawley and Witton, 1991; among others). Stockner (1991) indicates that concentrations of autotrophic picoplankton in several oligotrophic lakes in Canada becomes greater as the lake pH, conductivity, and productivity increases. However, as these lakes become eutrophic, there is also the accompanying increase in the biomass and productivity of the larger ( $>3\mu$ ) phytoplankton populations, so the percent contribution of the picoplankton to the total phytoplankton biomass and total photosynthesis decreases (Vörös et al., 1991; Burns and Stockner, 1991; Petersen, 1991). These larger phytoplankton taxa will proportionally represent the greater biomass and more primary producers than the picoplankton as the lake's eutrophic state increases. In contrast, the autotrophic picoplankton populations in oligotrophic lakes would be less abundant than in the eutrophic waters, but they would contribute a greater proportion to the lake's productivity and phytoplankton biomass than the less abundant and larger algal taxa. Picoplankton species within each of these lake types will typically be represented by both prokaryote and eukaryote taxa, but not the prochlorophytes. Coccioid cyanoprokaryote are typically ubiquitous, dominant in abundance, and may be represented by one or several taxa, or strains of a species (e.g. the *Synechococcus* complex). Depending on the investigators definition of picoplankton, colonial forms may also be included if the cells fall within the accepted size range. Peak development of these colonial taxa would be during the summer/early fall period, being more characteristic of eutrophic waters. Eukaryotes, are also ubiquitous, favoring cooler waters, and are more common in eutrophic and dystrophic lakes, with increased nutrients, and the pH  $<6.2$  (Stockner, 1991).

Weisse (1988), in a vertical assay of picoplankton in Lake Constance (a meso-eutrophic lake), reported concentrations up to  $10^5$  cells/mL, with the highest concentrations at 12-16 m depth, and then decreased with increased depth to  $10^4$  cells/mL at 140m.. Within this lake the cells were grazed actively by ciliates and heterotrophic nanoflagellates. Picoplankton was also reported in Lake Baikal by Boraas et al. (1991). Highest concentrations were at 5-10 m and decreased to 250m, with their abundance  $9.8 \times 10^4$  and  $4.2 \times 10^3$  cell/mL respectively, with mean water column values at  $2.7 \times 10^4$  cells/mL. The abundance of the prokaryotes in lakes has been correlated directly to temperature, with peaks occurring during summer and early fall (Caron et al., 1985). Many of the eukaryotes, and some of the prokaryotes, will also have an earlier bloom during mid- to late spring, followed by another bloom in late summer after stratification (Szelag-Wasilewska, 1997, 1999). These eukaryotes are typically 1.2-2.0 $\mu$  in size, and usually larger than most of the prokaryotes. Pick and Agbeti (1991) reported autotrophic cyanoprokaryote abundance peaks in oligo-mesotrophohic lakes in late summer at  $10^5$  cell/mL, and representing 1-9% of the phytoplankton biomass. The eukaryotes peak concentrations were less abundant ( $10^3$  cell/mL). The eukaryotes accounted for about half of the phytosynthetic picoplankton biomass in the "colored" lakes, and less than 20% in the clear water lakes. The major eukaryotes were *Chlorella* and *Nannochloris* spp. Exceptions to single summer periods for the cyanoprokaryote blooms have also been reported by Weisse and Kenter (1991), in Lake Constance. Over four consecutive years they noted spring and late summer blooms that were dominated by cyanobacteria. The range of abundance was  $10^2$  to  $10^6$  cells/mL, with the horizontal

differences across the lake as high as a factor of 3 in abundance and biomass during summer, and with more seasonal changes occurring in the upper eight meters of depth. They found differences in cell size seasonally, being larger in summer and fall, and with increasing depth (also Caron et al., 1985). Although the initiation and duration of these seasonal bloom periods may vary year to year among the various lakes, a typical pattern of abundance peaks during spring and late summer/early fall is common, as described by Szelag-Wasielewska (1998, 1999) in several Polish lakes. Decreasing abundance typically follows from late fall into winter and early spring.

Fahnenstiel and Carrick (1992) reported in Lakes Huron and Michigan that the autotrophic picoplankton was composed of 59% cyanobacteria and 21% eukaryotes with surface concentrations at  $10^3$  cells/mL. This represented 10% of the autotrophic plankton biomass with 17% of the primary production coming from the  $<1.0\mu$  fraction, and 40% from the  $<3\mu$  fraction. Picoplankton concentrations in Lake Ontario ranged from  $10^3$ - $10^5$  cells/mL (Pick and Caron, 1987). In a small shallow and oligo-mesotrophic lake in Poland, Szelag-Wasielewska (1999) reported the autotrophic picoplankton was dominated by cyanobacteria which had concentrations of  $10^5$ - $10^6$  cells/mL (these counts included colonial cyanobacteria). The dominant eukaryotes were species of *Chlorella* and *Choricystis*. Grazers included mixotrophic flagellates and ciliates. Wehr (1990) noted the development of pico- and nanoplankton were favored during summer periods of phosphorus limitation in a small eutrophic lake. Wehr (1991) also reports that most autotrophic picoplankton had greater biomass and abundance in phosphorus limiting systems, but were more influenced by nitrogen limitation, and seldom with phosphorus. However, Stockner and Shortreed (1989) noted in fertilized treated lakes, that the picoplankton abundance increased with added phosphorus. In Lake Tahoe, considered to be a phosphorus limited oligotrophic lake, the picoplankton represented 34-72% of the total productivity (Chang and Petersen, 1995). In another approach, evaluating the percent abundance of picoplankton to total algal biomass in 12 lakes of different trophic status, Szelag-Wasielewska (1997) found a negative relationship, with the range of abundance in these lakes from  $3.2 \times 10^3$  to  $1.16 \times 10^6$  cells/mL. In reference to what may happen to many of the picoplankton cells that settle out of the water column, Eguchi et al. (1996) found viable phototrophic picoplankton cells in the surface sediment of Lake Biwa (Japan). They indicated these may represent a seed population source that when re-suspended would produce further development of this population in the water column.

### PRODUCTIVITY

A major significance of the oceanic picoplankton community is their contribution to primary production (Gieskes et al., 1979). In studies within temperate coastal waters, this percentage has been commonly 20-30% (Larsson and Hagström, 1982; Joint et al., 1986), and in other estuaries up to 10% of the total production (Jochem, 1988; Ray et al., 1989). Iriarte and Purdie (1994), in waters of Southampton estuary reported autotrophic picoplankton abundance at  $10^4$  cells/mL, and that the  $<3\mu$  fraction was responsible for 17-20% of the total production, with the  $<1\mu$  fraction producing 6% of the total production. However, the highest values have been reported in the oligotrophic regions of oceans (e.g. up to 80% by Li et al., 1983; 77-82% by Takahashi and Bienfang, 1983)).



Teixeira and Gaeta (1991) estimated the autotrophic picoplankton (cells 0.45-1.0 $\mu$ ) productivity in the equatorial waters of Brazil in estuaries 3.0-28.5%, coastal regions 18.5-40.4 %, and oceanic 6.7-100%, of the total phytoplankton production. In the Southern ocean this percentage for the <1 $\mu$  fraction ranges seasonally 0-32% of the primary production (Weber and El Sayed, 1987). In an extensive review of primary production and abundance of autotrophic picoplankton (<3 $\mu$ ) in the Mediterranean Sea, Magazzu and Decembrini (1995) reported the abundance of the picocyanobacteria and the eukaryotes ranged from 10<sup>2</sup>-10<sup>5</sup> cells/mL, and the prochlorophytes at 10<sup>4</sup> cells/mL. The picoplankton productivity contribution was 44% and 71% for neritic and pelagic waters respectively. Bienfang et al. (1984) found in the tropical Pacific that the chlorophyll maximum area consisted mainly (60-80%) of cells < 3 $\mu$  , and that they represented 71% of the total production in the photic zone, and 77% of the chlorophyll. In sub-Antarctic waters, Vanucci and Mangoni (1999) reported the picoplankton was dominated by chroococcoid cyanobacteria (*Synechococcus* spp.), with the eukaryotes about one order of magnitude less abundant. Overall, in comparison to the total phytoplankton present, the picoplankton represented 46% of the chlorophyll and 53% of the primary productivity. In general, the autotrophic picoplankton represents up to 80% of the total primary production in marine waters.

In three tidal tributaries to the Chesapeake Bay, Marshall and Nesius (1993) reported the major phytoplankton peaks in productivity were enhanced by the autotrophic picoplankton when their peaks coincided with the summer productivity maximum. A similar pattern of a summer/fall enhancement of productivity by the increased picoplankton abundance was repeated over a four year period in the Chesapeake Bay (Marshall and Nesius, 1996). During the seasonal maxima the picoplankton concentrations in late summer and early fall were 10<sup>5</sup> and 10<sup>6</sup> cells/mL respectively. Within the southern Chesapeake Bay, the percent contribution of the picoplankton productivity to the total production during the spring/summer months of 2001 ranged from 6.4% (June) to 57% (July) (K. Nesius personal communication). The productivity rates for this period ranged from a river entrance site of 1.31- 28.46  $\mu$ gC/L/hr to 1.55 - 36.97  $\mu$ gC/L/hr at the Bay entrance. In their August diel study at the entrance to Chesapeake Bay, Affronti and Marshall (1993) reported the average picoproductivity rate above the pycnocline was 6.27 $\mu$ gC/L/hr, with lower productivity occurring in the morning, and below the pycnocline (0.77  $\mu$ g C/L/hr). In January, these productivity rates above and below the pycnocline were 0.134 and 0.153  $\mu$ gC/L/hr respectively. Using frequency of dividing cells to estimate growth rate and productivity at the Chesapeake Bay entrance, the picoplankton growth rates varied from 0.23/day to 1.10/day, with highest rates occurring in summer (Affronti and Marshall, 1994). These results indicate the picoplankton contribution to the total Bay productivity ranged from winter values of 2.2-2.3% to 53.4-55.6% in summer (July). Ray et al. (1989) determined a summer picoplankton production mean rate within a tidal river to be 2.5  $\mu$ gC/gChla/hr, and responsible for 9% total primary production, with a mean abundance of 2.75 X 10<sup>5</sup> cells/mL. In the northern, less saline, and more nutrient rich section of Chesapeake Bay, Malone et al. (1991) reported winter/spring productivity lows and summer highs, with picoplankton productivity rates over a two year period in August at 50-70  $\mu$ gC/L/hr, and a high the third year at 120  $\mu$ gC/L/hr. During the summer the picoplankton contributed 20% of the total production in this

area. In the Mediterranean, Magazzu and Decembrini (1995) had mean productivity rates within the coastal regions of  $1.19 \mu\text{gC/L/hr}$ , representing 31% of the total production, compared to the open water regions of  $1.73 \mu\text{gC/L/hr}$ , with a 92% contribution to total productivity. Picoplankton growth rates associated with the Great Lakes were 0.8-1.5/day, whereas in Pacific regions these varied from 0.97 to 3.62/day (Bienfang et al., 1984; Fahnenstiel et al., 1986; Bienfang and Takahashi, 1983; Iturriaga and Mitchell, 1986). Growth rates for *Prochlorococcus marinus* were reported by Moore et al. (1995) were from 0.53 to 0.63/day.

Numerous studies have also identified autotrophic picoplankton as major contributors to algal productivity in freshwater habitats (Rodhe et al., 1958; Holmes and Anderson, 1963; Kalff, 1972; Shiimoto et al., 1997; Steitz and Velinirov, 1999; Han and Furuya, 2000; among others). In the North American Great Lakes, Fahnenstiel et al. (1986) attributed 50% of the phytoplankton productivity in Lake Superior to cells  $<3\mu$  size, and were composed of 20% chroococcoid cyanobacteria at concentrations of  $10^3$  cells/mL. Of note, was that cells filtered through a  $<1.0\mu$  filter represented 20% of the primary productivity. In other studies, productivity rates within a variety of lakes of different eutrophic status were rather similar. These include the oligotrophic Great Central Lake ( $0.10\text{--}0.73 \text{ mgCm}^3\text{h}^{-1}$ ), Lake Superior ( $0.58 \text{ mgCm}^3\text{h}^{-1}$  and  $0.31 \text{ mgCm}^3\text{h}^{-1}$ ), and in eutrophic Lake Kinneret ( $0.01\text{--}1.5 \text{ mgCm}^3\text{h}^{-1}$ ) (Costella et al., 1979; Munawar and Fahnenstiel, 1982; Fahnenstiel et al., 1986; Malinsky-Rushansky et al., 1997).

#### TOXIC AND HARMFUL AUTOTROPHIC PICOPLANKTON

Although due to its colonial nature and community interactions, Komarek (1996) would exclude the *Microcystis* complex from the picoplankton category, strains within this group are known to be toxin producers (Hughes et al., 1958). They produce metabolites described as microcystins which are toxic to fish (Zimba et al., 2001; and others). Skulberg et al. (1993) and Codd (1995) makes reference to a variety of cyanobacteria as toxin producers that includes picoplankton strains within *Synechococcus* and *Synechocystis*. Toxicity associated with various strains of these genera are noted by Lincoln and Carmichael (1981) and Mitsui et al. (1989). These same two genera form a symbiotic association with several marine heterotrophic dinoflagellates (Gordon et al., 1994). The cells were found attached to the outer surface of these dinoflagellates in tropical and sub-tropical waters during periods of nitrogen limitation. Imai and Nishitani (2000) noted a similar relationship where unidentified picoplankton cells ( $1\text{--}2\mu$ ) were on the surface of the toxic marine dinoflagellates *Dinophysis acuminata* and *D. fortii*. These cells are a suggested food source for these heterotrophs and may be a source of toxicity in the *Dinophysis* spp. Glasgow and Burkholder (1998) found amoeboid and zooplankton stages of the toxic dinoflagellate *Pfiesteria piscicida* readily consumed *Synechococcus* cells. Similar uptake has been studied for these and other dinoflagellates by Marshall (unpublished), and others.

Since the mid-1980s, the chrysophyte *Aureococcus anophagefferens*, a spherical, unicellular organism ( $2\text{--}3\mu$ ), has produced annual blooms (called brown tides) in bays and inlets along the U.S. northeastern coast (Bricelj and Lonsdale, 1997). The blooms are associated with rising temperatures of spring ( $> 20^\circ\text{C}$ ), salinities  $>28 \text{ ‰}$ , and reduced flushing rates within these inlets associated with reduced spring rains. Bloom concentrations are in excess of  $1.0 \times 10^6$  cells/mL. The blooms coincide with the

growth season of *Zostera marina* producing extended periods of light attenuation to impact its development, and is associated with mass mortality among suspension feeding bivalves (mussels, bay scallops) by inhibition of their feeding. To date, no toxin has been linked to this species.

### PREDATION

There exist different opinions as to the relationships between the components of the microbial (food) loop and the predators in the metazoan food chain. Fogg (1995) considers the microbial loop more of a self-contained system that basically perpetuates itself by minimizing losses outside of the loop. Predation loss to metazoans is not extreme. Hagström et al. (1988) considers that only 6% of its biomass passes on to the higher trophic levels. Any losses outside the loop can be recaptured through nutrient enhancement from waste or decomposition products in the water column. Within this microbial loop, the microzooplankton predators would include protozoa, and a variety of mixotrophic and heterotrophic nanoflagellates (Caron et al., 1991; Kuuppo-Leinikki et al., 1994; Hadas et al., 1999; Sanders et al., 2000). The changing role of the autotrophic picoplankton is also directly related to trophic status. Weisse (1991) discusses the implications resulting from a shift from the smaller picoplankters to the larger eukaryotes and how this transition will be more beneficial to the metazoa. Safi and Hall (1999) used fluorescently labeled bacteria and microspheres as picophytoplankton sized particles to evaluate grazing by mixotrophic and heterotrophic nanoflagellates, and others. They found that the mixotrophic and heterotrophic nanoflagellates had a preference for the picoplankton sized particles over the bacteria when grazing on these artificial prey. The common nanoflagellates predators to picoplankton in oceans would include representative taxa within the pyrrhosiophytes, choanoflagellates, raphidophytes, dinoflagellates, chrysophytes, and euglenoids. Of note is that Cynar et al. (1985) report predaceous nanoplankton were present in both 0.4 and 0.6  $\mu$  water filtrates. In a study of herbivory in Newfoundland coastal waters, Putland (2000) found the microzooplankton consumed 25-30% of the *Synechococcus* standing crop daily. In the oligotrophic north Pacific, the coccoid cyanobacteria were the most abundant autotrophic picoplankton (64%) (Iturriaga and Mitchell, 1986). They estimated 30-40% of this cyanobacteria standing crop were consumed daily. The micro-grazers were represented by a diverse assemblage of protozoa, copepod larvae, and chaetognaths. The growth rates of these picoplankton cells was at 1.6/day.

Simek et al. (1995) studied protozoan grazing during summer within a eutrophic reservoir in Bohemia. The grazing rates were 560 picoplankton cells/hr for *Vorticella aquadulcis*. However, the dominant predators were oligotrichs, with an ingesting rate of 76-210 cells/hr. Other grazers of autotrophic picoplankton, and bacteria, included chrysomonads, choanoflagellates, ciliates, and bodonids. (Simek et al., 1997). At an oligo-mesotrophic lake, Pernthaler et al. (1996) identified the importance of heterotrophic nanoflagellates as grazers of picocyanobacteria and bacteria. They were responsible for ~90% of the grazing with the remaining 10% attributed to ciliates

The status of the picoplankton dominance within lakes will influence carbon utilization moving through the upper trophic levels. For instance, Stockner and Shortreed (1989) describe two contrasting oligotrophic lakes in British Columbia, which have different primary producers and predatory relationships that resulted in

trophic biomass differences. The picoplankton based food web results in a longer series of trophic steps to reach the higher trophic levels (e.g. fish). In contrast, the other lake has nanoplankton and microplankton phytoplankton as the primary producers, and is preyed in turn by larger zooplankton and reaches the fish consumption level in fewer steps, being more productive than the other lake. Considering the abundant and ubiquitous presence of picoplankton cells in these waters, Stockner (1988) considers a "top down" control of their abundance is very likely. This predation pressure appears to be caused mainly by various phytoflagellates and ciliates.

#### ANALYSIS OF AUTOTROPHIC PICOPLANKTON

There are significant limitations in light microscopy usage in the identification and enumeration of the various taxa within the picoplankton category. In addition to scanning electron microscopy and PCR analysis, two common approaches used today involve epifluorescence microscopy and flow cytometer analysis. References for these methods include: Davis and Sieburth (1982), Caron (1983), Wood et al. (1985), Pick and Caron (1987), Booth (1987), Weisse (1988), MacIsaac and Stockner (1993), Chisholm et al. (1988), Li and Wood (1988), Chisholm et al. (1992), Fahnenstiel et al. (1991), Olson et al. (1993), among others.

#### SUMMARY

Since the original identification of *Synechococcus* in the world oceans (Johnson and Sieburth, 1979; Waterbury et al., 1979), the composition of these ubiquitous picoplankton assemblages in both freshwater and marine locations has expanded. This has been accomplished through the utilization of more sophisticated instrumentation, along with the added scientific interest directed to this community over the past two decades. Although cyanobacteria are the most common and typically the more abundant representative within the picoplankton, there are a variety of eukaryote phylogenetic groups represented in both oceanic and freshwater habitats, with the prochlorophytes broadly distributed in the oceans. However, there remains questions as to what size groups should be included, and whether to exclude those colonial picoplankton from this category. These issues become important when attempting to compare data when investigators use different size categories to define their picoplankton, attribute productivity rates to this group, or if both prokaryotes and eukaryotes are included in studies.

In both freshwater and marine habitats the autotrophic picoplankton's percent contribution to the total primary production decreases moving from the less nutrient rich oligotrophic waters (e.g. off shore waters, open ocean) to the more eutrophic coastal regions (Craig, 1984; Fahnenstiel et al., 1986; Stockner, 1988; Stockner and Anita, 1986; Vörös et al. 1991; and Bell and Kalff, 2001). This occurs even as the picoplankton abundance increases into the more eutrophic waters. The major influence to this pattern is the greater concentrations of the larger eukaryotes ( $> 3\mu$ ) present in these more nutrient enriched waters in comparison to the picoplankton, in contrast to a reverse relationship at the oligotrophic sites. Their production is essential to the microzooplankton within the microbial loop in lakes and oceans, but linkages to predators that would bring this productivity to the higher trophic levels are limited and need further study and clarification.

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## Reproductive Differences among Delmarva Grass Shrimp (*Palaemonetes pugio* and *P. vulgaris*) Populations

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### ABSTRACT

Populations of female grass shrimps (*Palaemonetes pugio* and *P. vulgaris*) were sampled from five coastal embayments in Delaware, Maryland, and Virginia (Delmarva) and compared with respect to reproductive and life history attributes. We observed interspecific differences in timing of reproduction, carapace length, ratio of carapace length to total body length, body weight, clutch weight, clutch size, and egg volume. Onset of reproduction in *P. vulgaris* lagged behind *P. pugio*. Although there was no difference in the relationship between clutch size and carapace length for the two species, carapace length/total body length in *P. pugio* was greater than that in *P. vulgaris*. A multivariate analysis of variance indicated significant differences in carapace length, clutch weight, body weight, clutch size, and egg volume attributable to effects of species, population, and interactions between them. At all sites, *P. pugio* produced larger eggs than *P. vulgaris*. Although the two species did not differ in reproductive effort, both species exhibited increases in reproductive effort with latitude. Clutch size also tended to increase with latitude for both species. In populations where both species were abundant, adult females of *P. pugio* were longer and heavier and produced heavier egg masses comprised of fewer, larger eggs.

### INTRODUCTION

In a classic paper, Hutchinson (1961) raised the issue of how so many similar species are able to coexist in the plankton given the prediction, from the principle of competitive exclusion (Gause, 1934; Hardin, 1960), that one species should outcompete the others. Coexistence of similar species is exemplified by the "grass shrimps" *Palaemonetes pugio* Holthuis and *Palaemonetes vulgaris* (Say) that abound in marshes and bays of the Atlantic and Gulf coasts of North America. These two closely related species often co-occur in estuarine sites (Williams, 1984). Although both species exhibit similar distribution patterns across their geographic ranges, they exhibit differences in within-habitat usage. In previous studies, it has been shown that habitat partitioning in these species is a consequence of interspecific differences in physiological tolerances toward salinity (Thorpe and Hoss, 1975; Knowlton and Kirby, 1984;

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Knowlton and Schoen, 1984; Khan et al., 1995, 1997) and dissolved oxygen (Welsh, 1975), substrate and cover preferences (Arguin et al., 1989; Knowlton et al., 1994; Khan et al., 1995, 1997), and interference competition (Thorp, 1976). In the present study, we examine whether differences in reproductive strategies could also promote resource partitioning between *P. pugio* and *P. vulgaris* and how these differences are maintained across a range of environmental conditions.

There is some evidence to suggest that reproductive strategies differ between the two species. Chambers (1982) and Yan (1987) found that Massachusetts populations of *P. pugio* exhibit greater mean clutch size than *P. vulgaris* with the same reproductive effort (ratio of gonadal weight to body weight). Although seasonal breeding periods of *P. pugio* and *P. vulgaris* are thought to be similar (Knowlton, 1970), Hoffman (1980) observed that Delaware populations of *P. pugio* produced three or more clutches while *P. vulgaris* produced no more than two clutches within a single season.

Within a species, reproductive characteristics might be expected to vary according to season and geographic location due to differences in temperature, photoperiod, and salinity. Latitudinal clines in egg number have been observed in birds, fishes, and mammals such that clutch size increases with latitude (reviewed by Fleming and Gross, 1990). Such variation in clutch size may reflect differences in the growing season. Salinity may also influence clutch size in estuarine organisms. For example, Alon and Stancyk (1982) found that *P. pugio* fecundity increased with prolonged exposure to lower salinities.

The purposes of this study were to explore the extent to which *P. pugio* and differ reproductively and to examine these differences across a range of environmental conditions. Accordingly, we compared reproductive attributes of *P. pugio* and *P. vulgaris* from five marine and estuarine sites in Chesapeake and "outer" (Atlantic) bays of Virginia, Maryland, and Delaware, spanning a substantial salinity gradient and a wide range of latitude. We examined the effects of species and population level variation on reproductive characteristics. To determine broader geographical patterns in reproductive strategies, we compared results of this study of Delmarva populations with studies from populations in other regions.

## METHODS

*Palaemonetes pugio* and *P. vulgaris* populations were sampled during the breeding period (May, July, and September 1987) at five locations (Figure 1; see Knowlton et al., 1994 for details). Collecting locations were selected so that two pairs of Chesapeake and Atlantic sites occurred at similar latitudes and spanned a broad range of salinities. Values at Chesapeake sites ranged from about 12 ppt at Station 5 to about 25 ppt at Station 3 while those at both Atlantic sites (Stations 1 and 2) were about the same, averaging about 30 ppt (Figure 1, Appendix A). Collections at each site were timed to occur at roughly the same time of day and stage of tide (about two hours prior to low tide, Appendix A). Samples, obtained using long-handled D-frame dip nets, were preserved on site in 95% ethanol. At time of collection, salinity was measured with a hand-held refractometer, air and water temperatures with a pocket thermometer. Dissolved oxygen (mg/L) was determined using a modified Winkler method (Hach Chemical Co., 1977).

In the laboratory, we used a dissecting microscope to separate species per sample according to criteria in Holthuis (1952). Sex was determined by noting the form of the



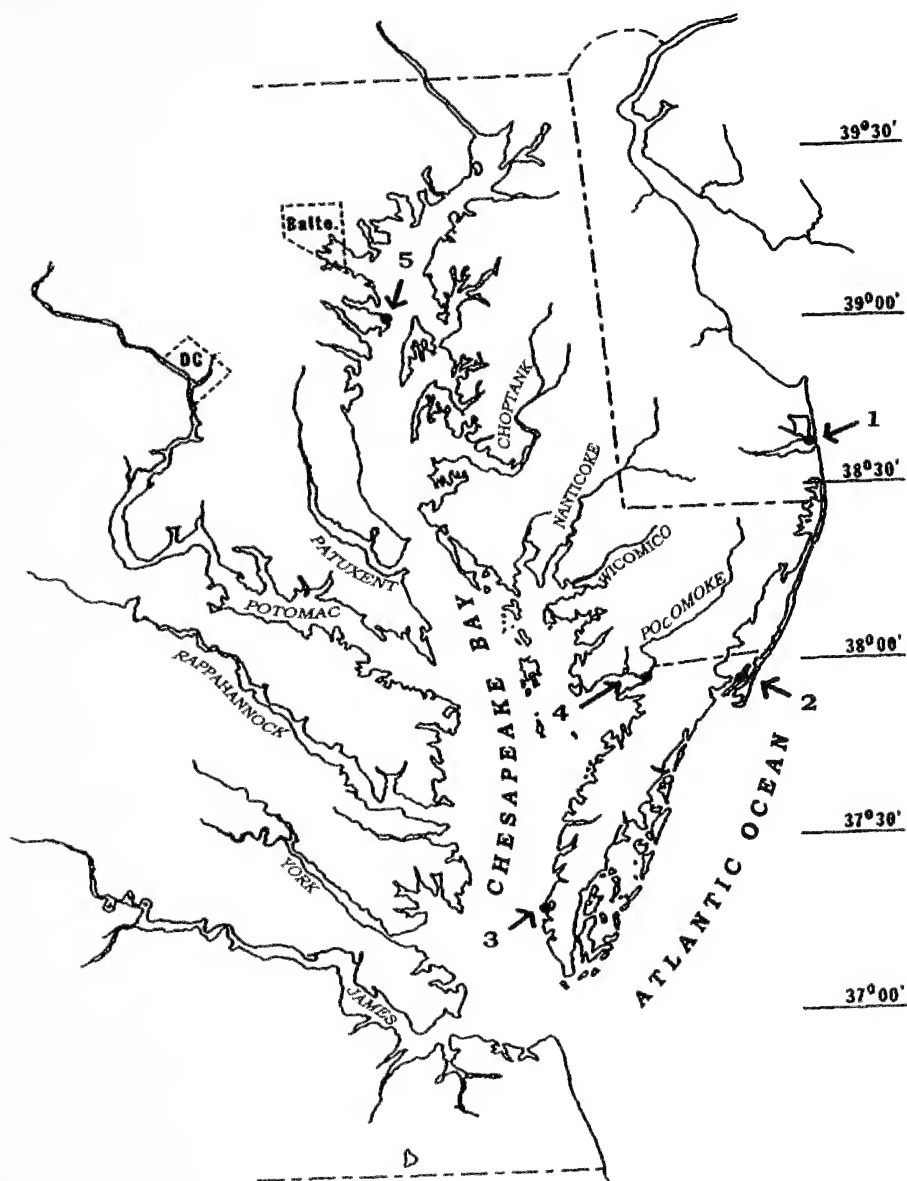


FIGURE 1. Locations of collection sites: 1 = Indian River Bay on Burton Island, DE; 2 = Chincoteague Bay, Chincoteague, VA; 3 = Kings Creek, Cape Charles, VA; 4 = Robin Hood Bay (of Pocomoke Sound), near Saxs, VA; and 5 = Mezick Pond, Sandy Point State Park, MD.

first pleopod endopod (shorter in female) or by the presence (male) or absence (female) of an *appendix masculina* on the endopod of the second pleopod (Williams, 1984). We also noted reproductive status of females (e. g., ovarian development) and percentage of females that were ovigerous. Body length (measured from tip of rostrum to posterior margin of telson) and carapace length (tip of rostrum to posterior end of carapace) of ovigerous females were measured with an ocular micrometer on the dissecting microscope. Length and width of stripped eggs were measured using the ocular micrometer. Body weight and egg weight were determined after shrimps and eggs were air dried for 48 hours at room temperature. In 203 individuals, all dried eggs found on each female were counted, but in 480 cases we extrapolated the number of eggs per female from subsamples of 100 eggs. Reproductive effort (RE) was calculated using Yan's (1987) "gonadosomatic index," as follows:

$$RE = G/(BW-G) \times 100$$

where G = dry weight of eggs and BW = dry body weight of ovigerous female. Egg volume was calculated using the formula for an ellipsoid as follows:

$$V = \frac{4 \pi abc}{3}$$

where a = width, b = height, and c = length (width and height were observed to be approximately the same). Amount of yolk (inversely proportional to embryonic development) and germinal disc size (larger with increased differentiation) were used to determine egg stage (described by Thomas, 1969) in each clutch.

Multivariate analysis of variance (ANOVA) was used to examine influence of species, population (station), and their interaction on female reproductive characteristics (Appendix B). After testing data for normality, we used the GLM (general linear model) procedure in SAS for a multivariate ANOVA with unbalanced design (SAS Institute, 1990). Where significant F-values were obtained from Type III Sum of Squares, means were compared using t-tests due to unequal sample sizes. Relationships between clutch size and carapace length, and between carapace length and body length were tested for linearity using least squares regression and Pearson's product-moment correlation (SAS Institute, 1990).

## RESULTS

Onset of reproduction in *P. vulgaris* lagged behind *P. pugio* in our study populations. In early May samples, *P. pugio* females bore eggs in early stages of development at all except northernmost stations (1 and 5). At the same time, almost all *P. vulgaris* females were non-ovigerous but had ripe ovaries (Table 1), indicating that a peak of egg deposition in this species will follow within 1-2 weeks. Observations of ovarian development, frequency of ovigerous females, and stage of egg development in these populations indicate that the reproductive season starts in late April-early May in *P. pugio*, mid-May in *P. vulgaris*, and continuing into late September in both species (Table 1).

The two species differed in body form but not in the relationship between clutch size and carapace length. Slopes of the regression lines depicting the relationship between carapace length and body length differed significantly, with *P. pugio* exhibiting a steeper slope than *P. vulgaris* ( $P < 0.05$ , Fig. 2). Although clutch size values tended to be lower in *P. pugio* than in *P. vulgaris*, the slopes of the regression lines for clutch size and carapace length did not differ significantly (Fig. 3).

TABLE 1. Timing of reproduction as indicated by percentage of ovigerous females (% OF) in samples and stage of egg development, as measured by % of the area occupied by yolk. Description of ovarian development is also provided as an indication of reproductive state in pre-reproductive females because ovaries ripen before eggs are deposited and brooded on the pleopods.

Date	Station	<i>P. pugio</i>		<i>P. vulgaris</i>		Comments
		% OF	Egg stage	% OF	Egg stage	
May 6	1	0	-	0	-	Ovaries ripe in both species
May 6	2	27.8	83	0	-	Eggs in early stages in <i>P. pugio</i> Ovaries ripe in <i>P. vulgaris</i>
May 7	3	50	62	0.5	80	Mostly early stages in <i>P. pugio</i> Ovaries ripe in <i>P. vulgaris</i>
May 7	4	33.3	95	0	-	Eggs in early stages in <i>P. pugio</i> Ovaries ripe in <i>P. vulgaris</i>
May 7	5	0	-	0	-	Ovaries ripe in <i>P. pugio</i>
July 2	1	81.3	38	81.8	42	Both species with eggs in all stages of development
July 2	2	50	75	85.2	57	100-50% yolk in <i>P. pugio</i> Eggs in all stages in <i>P. vulgaris</i>
July 3	3	29.3	48	82.3	45	Both species with all egg stages and juveniles present
July 3	4	84.9	18	75	40	All egg stages in both species
July 3	5	81.5	16	0	-	Both species with all egg stages and juveniles present
Sept. 12	1	0	-	0	-	Juveniles present in both species
Sept. 12	2	1.1	0	13.6	29	80-0% yolk in <i>P. vulgaris</i>
Sept. 13	3	7.4	69	0	-	100-40% yolk in <i>P. pugio</i>
Sept. 13	4	5.6	30	1.2	5	
Sept. 13	5	0	-	0	-	1 <i>P. pugio</i> juvenile present

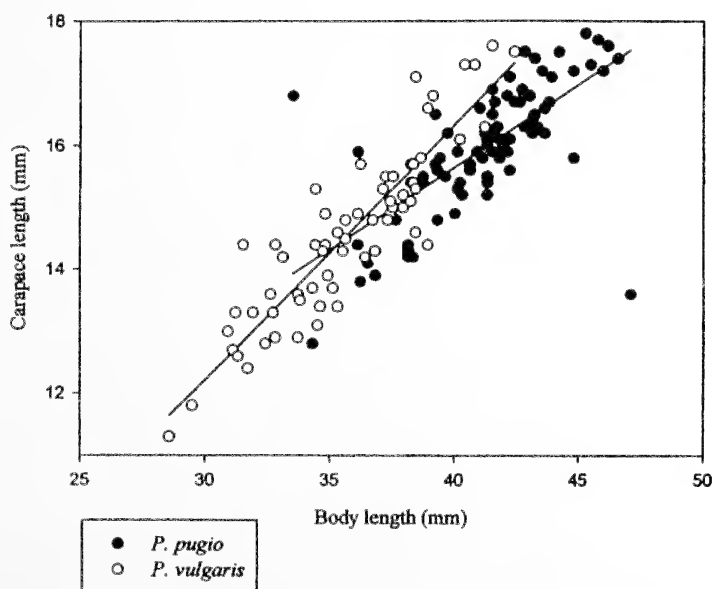


FIGURE 2. Morphological differentiation between female *Palaemonetes pugio* and *P. vulgaris*. Linear regression lines are included in graph; slopes of the regression lines for the two species differed significantly ( $P < 0.05$ ).

TABLE 2. Results of a multivariate analysis of variance for effects of species, station, and their interaction on grass shrimp reproductive attributes. F-values calculated using Type III Sum of Squares are listed with levels of significance indicated in parentheses. For all measurements, Station, Species, and Station x Species had 1, 4, and 3 Degrees of Freedom, respectively. Complete ANOVA tables are provided in Appendix B.

Measurement	Species	Station	Species x Station
Carapace length	13.94 (0.0003)	18.56 (<0.0001)	12.60 (<0.0001)
Body weight	19.00 (<0.0001)	26.40 (<0.0001)	9.95 (<0.0001)
Clutch size	16.68 (<0.0001)	41.04 (<0.0001)	3.99 (0.0092)
Clutch weight	8.39 (0.0044)	60.91 (<0.0001)	4.32 (0.0060)
Egg volume	39.22 (<0.0001)	0.84 (0.4998)	1.25 (0.2944)
Reproductive effort	0.47 (0.4940)	17.61 (<0.0001)	0.55 (0.6480)

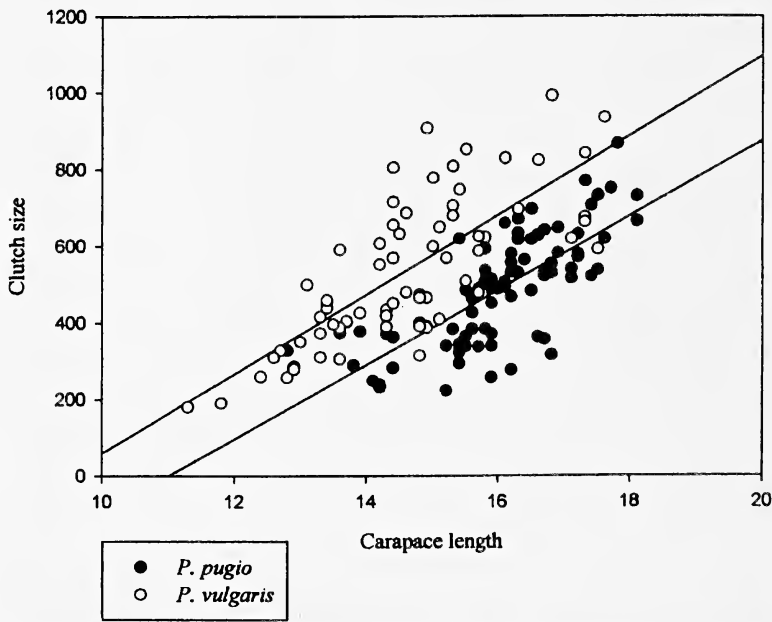


FIGURE 3. Relationship between clutch size and carapace length for *Palaemonetes pugio* and *P. vulgaris*. Slopes of the regression lines for the two species did not differ significantly.

Multivariate ANOVA indicated that variations in carapace length, body weight, clutch weight, and clutch size are significantly attributable to effects of species, population (station), and interactions between them (species x station; Table 2). Across all populations, *P. pugio* produced significantly larger eggs than *P. vulgaris* ( $t = 9.47$ ,  $P < 0.0001$ , Fig. 4). Reproductive effort in both species increased with increasing latitude (Fig. 5), and differences were significant between all stations except those that were adjacent oceanside (1 and 2) or bayside (3 and 4) (Table 3).

Significant first-order interactions occurred between species and population for the following features: carapace length, body weight, clutch weight, and clutch size (Table 2). Therefore, we restricted means analyses to comparisons of the two species to populations where we obtained sufficient sample sizes of ovigerous females of both species (Station 1: *P. pugio*,  $n = 21$ , *P. vulgaris*,  $n = 23$ ; Station 3: *P. pugio*,  $n = 16$ ,

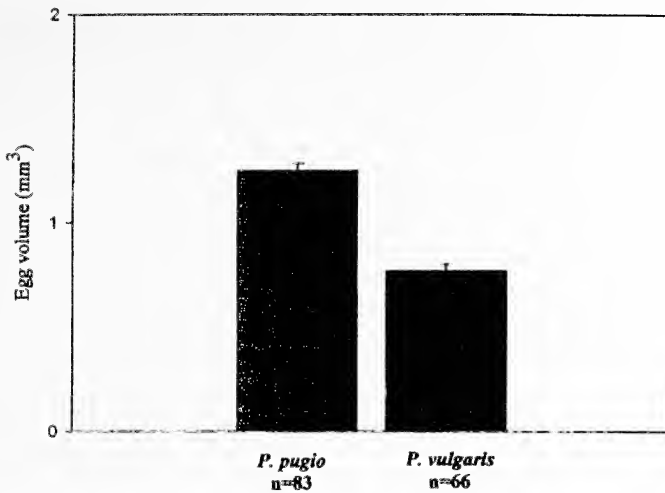


FIGURE 4. Interspecific variation in egg volume. Volume of *Palaemonetes pugio* eggs was 162% greater (mean  $\pm$  standard error;  $1.2461 \pm 0.0369 \text{ mm}^3$ ) than that of *P. vulgaris* eggs ( $0.7678 \pm 0.0323 \text{ mm}^3$ ).

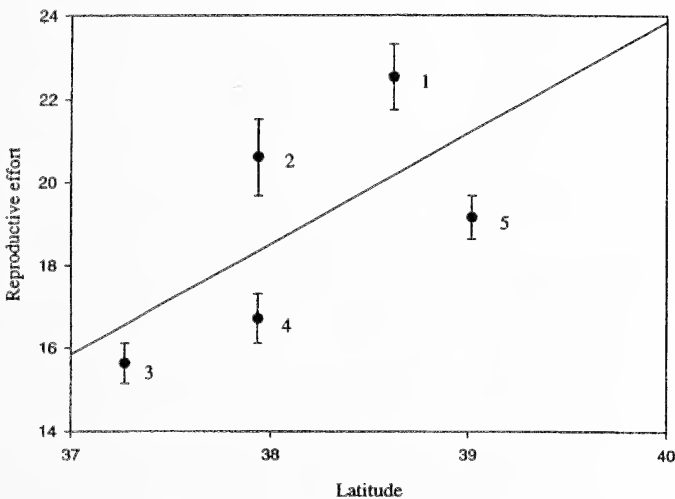


FIGURE 5. Latitudinal variation in reproductive effort for *Palaemonetes pugio* and *P. vulgaris* combined, with regression line fitted to means (adjacent number indicates station); each vertical bar indicates standard error.

*P. vulgaris*,  $n = 20$ ). Station 2 was dominated by *P. vulgaris*, Stations 4 and 5 by *P. pugio*; these differences in distribution patterns are thought to arise from different salinity tolerances exhibited by the two species (Knowlton et al., 1994). *Palaemonetes pugio* exhibited mean carapace lengths 1.12-1.16 times greater than in *P. vulgaris* (Station 1:  $t = 6.0937$ ,  $P < 0.0001$ ; Station 3:  $t = 6.7855$ ,  $P < 0.0001$ ). We observed similar trends in dry body weight measurements, *P. pugio* being 1.43-1.58 times heavier than *P. vulgaris* (Station 1:  $t = 7.6324$ ,  $P < 0.0001$ ; Station 3:  $t = 5.9727$ ,  $P < 0.0001$ ). Mean clutch weights were 1.31-1.40 times greater in *P. pugio* than in *P. vulgaris* (Station 1:  $t = 5.5982$ ,  $P < 0.0001$ ; Station 3:  $t = 3.0025$ ,  $P < 0.004$ ). At

TABLE 3. Results of t-tests comparing reproductive effort among populations occurring at Stations 1-4, expressed as t values, with levels of significance indicated in parentheses. Station 5 was not included in the means analyses because only one species (*P. pugio*) was found there.

	Station		
	1	2	3
2	1.5763 (0.1172)		
3	7.8075 (<0.0001)	4.2909 (<0.0001)	
4	4.4620 (<0.0001)	2.7050 (< 0.0100)	0.5952 (0.5526)

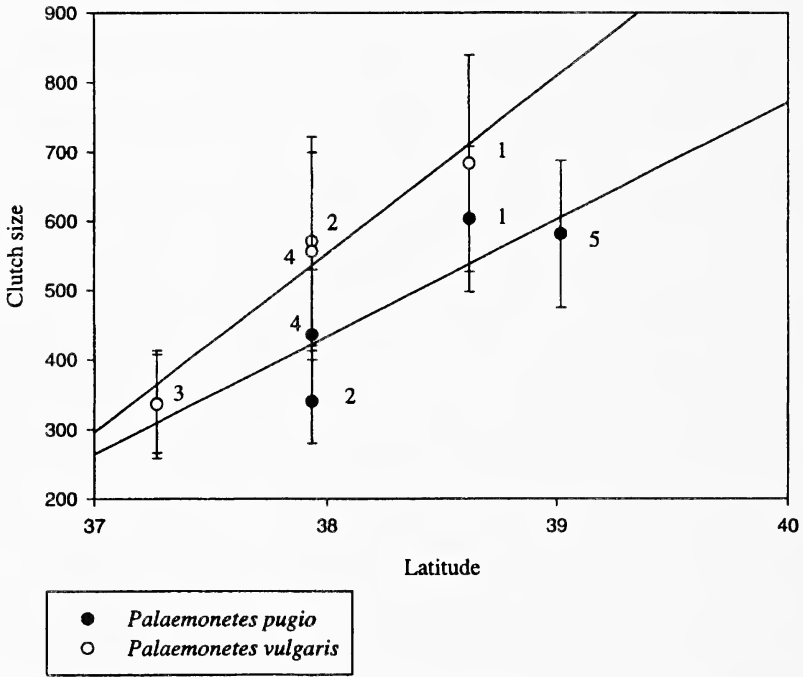


FIGURE 6. Latitudinal variation in clutch size for *Palaemonetes pugio* and *P. vulgaris*, with regression line fitted to means (adjacent number indicates station); each vertical bar indicates standard error.

Station 1, *P. vulgaris* mean clutch size was 1.13 times larger than that of *P. pugio* ( $t = 2.3165$ ,  $P < 0.025$ ). No significant difference in clutch size was detected between the two species at Station 3 ( $t = 0.0325$ ,  $P = 0.9741$ ). Both species exhibited a trend of increasing clutch size with latitude (Fig. 6).

### DISCUSSION

Populations of *P. pugio* and *P. vulgaris* occurring in Delmarva bays differed significantly in a number of reproductive attributes that may correspond to differences in reproductive strategies. Between populations where both species were abundant, *P. pugio* was determined to be larger and heavier, producing clutches that were heavier but sometimes smaller in egg number, compared with *P. vulgaris*. The breeding season in *P. pugio* lasted approximately two weeks longer than in *P. vulgaris* in the populations

TABLE 4. Length of breeding seasons of *Palaemonetes pugio* and *P. vulgaris* at different coastal locations.

Location	Species	Breeding season (weeks)	Authority
Woods Hole, MA	both	June to late August (10)	Faxon, 1879; Jenner, 1955
Narragansett Bay, RI	<i>P. pugio</i>	May to late July (10)	Welsh, 1975
Canary Creek, DE	<i>P. pugio</i>	mid-May to early September (16)	Hoffman, 1980
	<i>P. vulgaris</i>	late-May to early September (15)	
Cape Charles, VA	<i>P. pugio</i>	late April to late September (22)	this study
	<i>P. vulgaris</i>	early May to late September (20)	
Bogue Sound, NC	both	early April to mid- October (28)	Broad, 1957; Knowlton and Williams, 1970
North Inlet, SC	<i>P. pugio</i>	late March to mid- October (29)	Alon and Stancyk, 1982
Galveston Bay, TX	<i>P. pugio</i>	March to October (31)	Wood, 1967

we studied (Table 4). Differences in onset of reproduction between these species may allow *P. pugio* to produce more broods per season than *P. vulgaris*. In Delaware, Hoffman (1980) observed that *P. pugio* began spawning in mid-May and produced three or more broods per season while *P. vulgaris* began spawning in late May and produced only two broods. Our study indicates that differences in breeding season between these species may occur across populations in the Delmarva region. Such differences may also serve to reduce competition among planktonic zoea larvae, which are morphologically very similar (Broad, 1957).

In all populations, eggs of *P. pugio* were larger than those of *P. vulgaris*. Increasing offspring size is a reproductive strategy that may increase offspring fitness. In plants, large seeds can be expected to have selective advantage, and increases in egg size in animals are positively correlated with hatching success, survival, tolerance of starvation, size at hatching, and growth (Roff, 1992). Within the Palaemoninae, there is evidence that egg size increases starvation tolerance. For example, Mashiko (1985) determined that larvae (of the freshwater shrimp, *Palaemon paucidens*) hatched from larger eggs were significantly more tolerant of starvation than those hatched from smaller eggs.

Latitudinal trends in clutch size and reproductive effort were apparent in the mid-Atlantic populations we studied. Both species exhibited increases in clutch size with latitude (Fig. 6). Fleming and Gross (1990) found that latitudinal patterns in clutch size in Pacific salmon arose from a negative relationship between latitude and egg size. We did not observe any latitudinal trend in egg size in our study populations. Instead, we found that reproductive effort increased with latitude for both *P. pugio* and *P. vulgaris* (Fig. 5).

Reproductive characteristics may vary with latitude in response to differences in the length of the breeding season. Breeding in *Palaemonetes* spp. is dependent on photoperiod and temperature (Little, 1968). A pattern of shorter breeding season with

TABLE 5. Comparison of reproductive characteristics between Delmarva and Massachusetts populations of *Palaemonetes pugio* and *P. vulgaris*. Mean  $\pm$  standard deviation for Delmarva populations sampled July 1987 (present study); means for Massachusetts populations sampled July 1984 (Yan 1987).

Characteristic	<i>Palaemonetes pugio</i>		<i>Palaemonetes vulgaris</i>	
	Delmarva	Massachusetts	Delmarva	Massachusetts
Carapace length (mm)	16.0 $\pm$ 1.1	8.7	14.6 $\pm$ 1.4	7.1
Clutch size	487 $\pm$ 143	590	542 $\pm$ 194	330
Clutch weight ( $\mu$ g)	53.3 $\pm$ 7.9	62.9	38.6 $\pm$ 4.2	56.1
Reproductive effort (%)	18.4 $\pm$ 4.3	22.8	21 $\pm$ 5.3	22.9

increasing latitude in *P. pugio* and *P. vulgaris* is clear when our results are combined with data in the literature (Table 4). The observed increases in reproductive effort with latitude in *P. pugio* and *P. vulgaris* may be a consequence of more northern populations compensating for shorter breeding seasons. Other abiotic factors may also influence expression of life history traits. Alon and Stancyk (1982) attributed differences in life history traits of two different South Carolina populations of *P. pugio* to effects of salinity and other environmental variables. Although we did not sample enough populations to differentiate between effects of salinity and latitude, we found that outer bay (Atlantic) populations exhibited greater reproductive effort at similar latitudes than inner bay (Chesapeake) populations (Fig. 5). Both species exhibited a significantly greater reproductive effort at Station 2 (Atlantic) than Station 4 (Chesapeake) despite close latitudinal proximity (Table 3).

In Massachusetts populations of *P. pugio* and *P. vulgaris*, Yan (1987) found that the former species had a longer carapace length and produced a heavier clutch with no greater reproductive effort (Table 5). In contrast to our results on Delmarva populations, Yan (1987) determined that the Massachusetts population of *P. pugio* produced larger clutches than *P. vulgaris*. This difference apparently arises from higher fecundity of *P. pugio* and lower fecundity of *P. vulgaris* in the Massachusetts population than in Delmarva populations. For both species, reproductive effort was slightly higher and carapace length was shorter in the Massachusetts population (Table 5). Although Yan (1987) did not measure egg size directly, the sample population of *P. vulgaris* from Massachusetts produced a heavier clutch comprised of fewer eggs, which suggests that the eggs were heavier and may be larger than in Delmarva populations. No such trend in *P. pugio* egg size was seen between the two populations.

Yan (1987) concluded that *P. pugio* produces more offspring than *P. vulgaris* in order to compensate for greater exposure to predation pressure. Thorp (1976) suggested that there might be differences in relative predation pressure between *P. pugio* and *P. vulgaris* when he determined that *P. vulgaris* displaces *P. pugio* from preferred and more protected strata (e.g., shell) to mud. Increased predation pressure is expected to select for earlier onset of reproductive maturity (Gadgil and Bossert, 1970) if there are no indirect effects of predation on prey food supply (Abrams and Rowe, 1996). If there are such indirect effects, increased predation pressure will have the opposite effect and predation can select for delayed maturity and increased body size (Kawecki, 1993; Abrams and Rowe, 1996). However, investigation of possible predator or prey effects on life history traits requires an experimental approach.



In our study, we observed substantial differences in life history traits within and between two closely related species, *P. pugio* and *P. vulgaris*. Both species exhibited geographical variations that may reflect plasticity in life history traits or adaptation to local conditions. Future experiments, in which animals from widely separated sites are maintained under controlled environmental conditions, will elucidate whether these differences reflect plasticity or fixed differences among populations. We also observed interspecific differences in life history traits, summarized as follows: adults of *P. pugio* began reproducing earlier in the season and were longer and heavier, producing an egg mass with fewer, larger eggs than those of *P. vulgaris*. Physiological and behavioral differences have been regarded as important factors promoting resource partitioning between two closely related species that are frequently sympatric. Our study provides evidence that interspecific variation in timing of reproduction and other elements of reproductive strategy are also important.

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APPENDIX A. Abiotic parameters measured at collecting stations. Each station included two study sites: A = a marina consisting of wooden pilings, buttresses, docks, etc. B = nearby sand, mud, or oyster shell flats typically bordered by marsh vegetation (*Spartina alterniflora* at Stations 1-4, *Phragmites australis* at Station 5).

Month	Station	Time	Salinity (ppt)	Air temp. (°C)	Water temp. (°C)	Dissolved O <sub>2</sub> (mg/l)
May	1A	1550	24	21	17	9.9
	1B	1610	27	20	13	9.5
	2A	1830	28	19	14	8.8
	2B	1855	22	18	15	8.7
	3A	0850	20.5	14	16	4.7
	3B	0910	21	16	16	9.0
	4A	1115	15.5	19.5	16	6.6
	4B	1130	16	19	16	7.8
	5A	1530	9	20	18	9.5
July	5B	1555	9	22.5	19	10.7
	1A	1405	33.5	29	23.5	7.2
	1B	1450	33	32	22	7.2
	2A	1720	33.5	26	23.5	10.1
	2B	1745	33	24	25	11.4
	3A	0620	25.5	24.5	26	4.0
	3B	0645	24.5	24	25.5	3.3
	4A	1030	17	29.5	26	4.7
	4B	1105	16.5	28	26	7.3
September	5A	1520	11	31	29	7.3
	5B	1555	11.5	33	29.5	7.4
	1A	1350	32	26	25	6.7
	1B	1435	32	24	24	7.0
	2A	1700	33	25	24.5	6.8
	2B	1740	32.5	24	24	5.1
	3A	0605	27.5	22	24	4.3
	3B	0625	27.5	22	24	4.5
	4A	1030	17	22	23	2.5
	4B	1050	17.5	21.5	23	5.7
	5A	1515	12.5	24	25	8.5
	5B	1535	12	23.5	23.5	5.5

APPENDIX B. More detailed tabulation of results (summarized in Table 2) of multivariate ANOVA pertaining to effects of station, species (*Palaemonetes pugio* and *P. vulgaris*), and their interaction (Station\*Species) on reproductive attributes.

## Carapace length

Source	DF	Type III SS	Mean Square	F Value	P > F
Station	4	65.82136235	16.45534059	18.56	<0.0001
Species	1	12.35722881	12.35722881	13.94	0.0003
Station*Species	3	33.50236478	11.16745493	12.60	<0.0001

## Body weight

Source	DF	Type III SS	Mean Square	F Value	P > F
Station	4	0.05466830	0.01366708	26.40	<0.0001
Species	1	0.00983553	0.00983553	19.00	<0.0001
Station*Species	3	0.01545745	0.00515248	9.95	<0.0001

## Clutch size

Source	DF	Type III SS	Mean Square	F Value	P > F
Station	4	2131635.441	532908.860	41.04	<0.0001
Species	1	216630.862	216630.862	16.68	<0.0001
Station*Species	3	155335.574	51778.525	3.99	0.0092

## Clutch weight

Source	DF	Type III SS	Mean Square	F Value	P > F
Station	4	0.00635852	0.00158963	60.91	<0.0001
Species	1	0.00021906	0.00021906	8.39	0.0044
Station*Species	3	0.00033843	0.00011281	4.32	0.0060

## Egg volume

Source	DF	Type III SS	Mean Square	F Value	P > F
Station	4	0.31408827	0.07852207	0.84	0.4998
Species	1	3.65023949	3.65023949	39.22	<0.0001
Station*Species	3	0.34877494	0.11625831	1.25	0.2944

## Reproductive effort

Source	DF	Type III SS	Mean Square	F Value	P > F
Station	4	1110.816493	277.704123	17.61	<0.0001
Species	1	7.417646	7.417646	0.47	0.4940
Station*Species	3	26.095286	8.698429	0.55	0.6480







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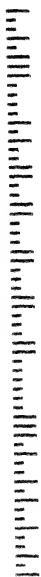
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# PROCEEDINGS

## ABSTRACTS OF PAPERS, 80th Annual Meeting of the Virginia Academy of Science, May 22-24, 2002, Hampton University, Hampton, Virginia

### Aeronautical and Aerospace Sciences

(No Abstracts Submitted)

### Agriculture, Forestry and Aquaculture Science

ROW SPACING MANIPULATION AND GWOP CANOLA. Harbans L. Bhardwaj, Agricultural Research Station, Virginia State University, Petersburg, VA 23806. GWOP (Grown With Out Pesticides) canola (*Brassica napus*) has the potential to establish canola as an alternative crop for supplying healthy edible oil. Canola is generally planted in rows spaced 15 to 20 cm. This practice, precludes the use of mechanical cultivation and necessitates the use of herbicides for weed control. If canola could be produced by planting in wider rows without reducing the oil yield and quality, it would allow mechanical cultivation and facilitate organic production of canola. The results of a replicated field experiment, conducted during 2000-2001 season, indicated lack of statistical differences for seed yield, oil content in the seed, and oil yield for 15, 30, 45, 60, 75, and 90 cm row spacings (2898, 2413, 1943, 2180, 1963, and 2223 kg/ha; 38.5, 39.4, 40.0, 41.1, 39.4, and 40.1 percent; and 1106, 943, 776, 896, 771, 890 kg/ha, respectively). The fatty acid profiles were also not affected by row spacings, the total saturated fatty acids varied from 8.6 to 9.8 percent, total unsaturated varied from 90.2 to 91.4 percent, mono-unsaturated fatty acids varied from 65.1 to 67.2 percent, and poly-unsaturated fatty acids varied from 23.0 to 26.5 percent. The results indicated that canola can be grown by using any of the six row spacings evaluated in this study. Significance of these results lies in the fact that canola can be produced organically by using wide rows.

SULFUR FERTILIZER RATE EFFECTS ON CANOLA YIELD AND QUALITY. Ronald A. Bowen and Harbans L. Bhardwaj, Agricultural Research Station, Virginia State University, Petersburg, VA 23806. Efforts by Virginia State University (H.L. Bhardwaj) and Virginia Tech (D.E. Starner) have led to development of a canola production system which consists of planting in late September to middle October, use of 5-6 kg/ha seed, and use of 100 kg/ha each of nitrogen, phosphorus, and potassium. These efforts have also developed a new cultivar (VSX-1) which is adapted to this region. However, the information about use of sulfur fertilizer in canola production in Virginia is not available. A replicated field experiment during 2000-2001 season indicated that the sulfur fertilizer rate significantly affected the seed yield which varied from 1734, 1765, 2209, and 2705 kg/ha, respectively for 0, 15, 30, and 45 kg S/ha. The S rate effects on oil content were not significant but because of significant effects on seed yield, the oil yield was significantly affected by S fertilizer rates. The oil yield varied from 724, 745, 930, and 1097 kg/ha, respectively for 0, 15, 30, and 45 kg S/ha. The oil yield following 30 and 45 kg S/ha were statistically similar and were statistically superior to those following 0 and 15 kg S/ha. Effects of S fertilizer rates on fatty acid composition of oil were not significant. These results indicate that it may be desirable to apply 30 kg/ha of sulfur for highest canola oil yield in Virginia.

CHARACTERIZATION OF THE CHLORIDE CHANNEL-1 (CLC-1) GENE IN SHEEP WITH CONGENITAL MYOTONIA. Altamarie Woods & Brian L. Sayre, Agriculture Research Station, Virginia State University, Petersburg 23806. Myotonia is a condition that causes delayed relaxation of muscles after sudden contractions. In Myotonic goats, myotonia was caused by a single-point mutation in CLC-1 gene. This condition has also been observed in some sheep. The objective of this study was to determine if myotonic sheep had the same CLC-1 mutation. Jugular blood was collected from sheep that expressed the myotonic phenotype (myotonic; n = 3) and did not express the

myotonic phenotype (normal; n = 4). Genomic DNA was extracted from whole blood samples. A 177-bp fragment, that contained the *CLC-1* mutation in goat, was amplified by PCR, and products were subjected to Mbo II restriction enzyme digestion. After Mbo II digestion, two fragments (105 and 72 bp) were found, similar to that found in normal goats. There were no differences in the digested fragment pattern between normal and myotonic sheep. The single-point mutation related to myotonia in the goat was apparently not present in the myotonic sheep. Further characterization of the *CLC-1* gene in the myotonic sheep may result in the discovery of a different mutation that leads to the myotonic phenotype, and further our understanding of congenital myotonia in animals and humans.

TWO YEAR SUMMARY OF GOLDEN TROUT IN WINTER CAGE CULTURE. Scott H. Newton, Cooperative Extension, Virginia State University, Petersburg, VA 23806. Golden trout, a genetic color variation of rainbow trout, were reared in side by side cage trials with rainbow during the fall to spring seasons of 2000-2001 and 2001-2002. Golden trout survival was 91% and 85% while rainbow trout survival was 92% and 79%, respectively for the two seasons. Average overall weight gains and feed efficiencies between the two strains were not significantly different. Therefore, it appears at this point that selection of which strain to produce may be more closely related with marketing and sales than rather than significant production differences. A third season of production comparisons between golden and rainbow trout will be conducted during the fall to spring season of 2002-2003.

IMPORTANT STEPS IN AQUACULTURE NUTRITION RESEARCH. Craig S. Kasper, Virginia Cooperative Extension, Virginia State University, Petersburg VA 23806. Aquaculture production must expand to keep up with increasing demand for fish. Increasing aquaculture production will provide food, alternative markets for agriculture products and supplement stocks used in recreational fisheries. Fish nutrition is of critical importance to aquaculture production, especially when confronted with new species. Determining natural food preferences will help to facilitate feed production and stimulate industry growth. Practical diets formulated from known nutrient requirements and manufactured utilizing low-cost, readily available oilseeds, can help to reduce variable costs of feed in fish production by conserving expensive fish meal and increase aquacultural production while improving environmental quality and fish health.

PRELIMINARY FINDINGS ON EFFECTS OF ENERGY TO PROTEIN RATIOS IN YELLOW PERCH (*Perca flavescens*) S.R. Craig<sup>1</sup> and E. McLean<sup>2</sup> <sup>1</sup>Virginia/Maryland Regional College of Veterinary Medicine, VPI&SU and <sup>2</sup>Department of Fisheries & Wildlife Sciences, VPI&SU. Results from a preliminary trial investigating the effects of different protein to energy ratios on weight gain and biological indices of yellow perch (*Perca flavescens*) was performed in a recirculating system consisting of 10 gallon glass aquaria. Experimental diets with energy to protein ratios ranging from 6 to 14 were fed to triplicate groups of yellow perch (n=8/aquaria) for 10 weeks. Yellow perch fed the diets containing the highest energy to protein ratios (11.2 and 14.8) had significantly reduced weight gain compared with fish fed the diets with energy to protein ratios under 10, which all had similar weight gain. Biological indices measured included hepatosomatic index (HSI), intraperitoneal fat (IPF) ratio and visceral mass index. No significant dietary effects were observed on the biological indices measured, although yellow perch fed the diets with higher energy to protein ratios tended to have larger HSI. All fish in the present study had significant lipid deposition as measured by the IPF ratio, with fish in all treatments having at least an IPF ratio of 4 or higher. Further refinement of the energy to protein ratio in this species is warranted.

PRELIMINARY RESULTS OF SOUTHERN FLOUNDER (*Paralichthys lethostigma*) FRY PRODUCTION IN RECIRCULATING AQUACULTURE SYSTEMS (RAS). M. H. Schwarz<sup>1</sup>, R.W. Cool<sup>1</sup>, D.E. Mowry<sup>1</sup>, S.R. Craig<sup>2</sup>, E. McLean<sup>3</sup>, and M.L. Jahncke<sup>1</sup>. <sup>1</sup>VSAREC 102 S. King



Street, Hampton VA, 23669, <sup>2</sup>College of Veterinary Medicine, VPI&SU, and <sup>3</sup>Department of Fisheries & Wildlife Sciences, VPI&SU. Results from the preliminary trial of southern flounder fry production in RAS at the Virginia Seafood Agricultural Research and Extension Center (VSAREC) were positive. From 19,000 eggs, 15,000 southern flounder fry were hatched. Upon hatching, fry were placed into a "greenwater" RAS. Greenwater production techniques included: 1) larval fish densities of 30/ml, 2) live algal densities (a combination of *Nannochloropsis* sp., *Isochrysis* sp., and *Tetraselmis* sp.) of 300,000 cells/ml, 3) rotifer densities (*Brachionus* sp.) in excess of 15/ml, and 4) artemia (*Chirocephalus* sp.) densities of 2-4/ml. After placement into the greenwater system (one day post-hatch) the fry quickly grew to metamorphosis by day 40, at which time they were placed onto commercially available dry feeds. At metamorphosis, a survival rate of 73% from hatching was observed. A secondary trial of fry production under similar protocols is presently under way with summer flounder (*Paralichthys dentatus*).

REVIEW OF BARLEY STRAW FOR ALGAE CONTROL. Brian L. Nerrie, Coop. Ext., Virginia State Univ., Petersburg, VA 23806. Algae are one of the primary producers in the aquatic environment, serving as a food source and provider of dissolved oxygen. However, excess algae can be problematic, impacting on aesthetics, water quality, and the valued uses of the aquatic resource. Aquatic managers are requesting new alternative algae control methods. Chemical herbicides are still widely used, but can result in water use restrictions. Overuse of chemicals is a concern. Copper, the most widely used herbicide ingredient for controlling algae, is relatively safe, but under low alkalinity conditions, can be highly toxic to fish, especially trout. Barley straw was shown during the 1990s to inhibit algae growth in Great Britain. Hydrogen peroxide, one of the products of decay, is suspected of being the algae inhibitor. Barley straw has been adopted by water gardeners in the United States for use as an algae control agent. Managers of ponds and lakes are examining its potential. Proper estimates of surface area are necessary. Barley straw is packed loosely into sacks at 90-225 kg/ha. Straw sacks are maintained in the top meter of water allowing flow through the straw. The straw is effective for up to 6 months at water temperatures exceeding 20° C. The Virginia Fish Farmers Association and Virginia State University are conducting a SARE funded study using barley straw.

IMPACT OF FEEDING ON DISSOLVED OXYGEN CONCENTRATION IN RAINBOW TROUT CAGE CULTURE. Tony J. Robinson & Brian L. Nerrie, Dept. Agriculture and Home Ecology, Virginia State Univ., Petersburg, VA 23806. Several Virginia fish farmers are double cropping ponds by feeding warm water fish (catfish, hybrid striped bass) during warm water temperatures and cold water fish (trout) during cold water temperatures. Cold water fish such as rainbow trout (*Onchorhynchus mykiss*) cannot survive temperatures that exceed 22° C for extended periods of time. Cage culture is a production technique by which fish are stocked at high density and fed a complete diet in a cage floating just below the surface. Rainbow trout were stocked at 300 fish per 1.7 m<sup>3</sup> cage in cages located in an aerated 0.05-ha pond at Virginia State University's Randolph Farm Aquaculture Facility during the winter growing season of 2001-2002. Cages were constructed from 1.25-cm mesh plastic mesh. This study examined the impact of feeding activity on the dissolved oxygen concentration (D.O.) inside the trout cage. Trout were offered a 4-mm floating trout pellet at 1% body weight. D.O. measurements were taken at the surface and bottom of the cage before feed was offered and 15 minutes post-feeding. Water quality parameters (alkalinity, hardness, and temperature) were maintained within the range for trout production. Feed activity reduced the D.O. inside the cage surface water by an average of 9.5 % following initial feed consumption.

HIGH DENSITY INTENSIVE ROTIFER CULTURE FOR THE FIRST FEED STAGE OF JUVENILE SOUTHERN FLOUNDER (*Paralichthys lethostigma*). R. W. Cool, M. H. Schwarz, M. L. Jahncke, D. E. Mowry, Virginia Seafood Agricultural Research and Extension Center (VSAREC), 102 S. King Street, Hampton, VA 23669. As with any finfish, the first-feed stage of juvenile southern flounder is the most critical period in the fish's life. The southern flounder is a sight

feeder with very little propulsion skills. They rely on aeration currents to move throughout the system as juveniles. Adequate feed in a system is a crucial requirement to fulfill the nutritional needs of these juvenile fish in their pre-metamorphosis stage. The first live feed for the juvenile southern flounder is the marine rotifer (*Brachionus plicatilis*), an aquatic zooplankton, which can be challenging to maintain at required densities. Intensive, high-density rotifer culture is the backbone for high survival rates of first-feed stage larval fish. Culture parameters and techniques need to be easily maintained to produce ~1,000 rotifers/mL during spawning season. Maintaining 15-30 rotifers/mL in a larval system can be a tedious chore with the voracious feeding characteristics of flounder. High-density rotifer cultures are a necessity to fulfill the critical weaning stage and attain high survival rates with juvenile flounder.

**AQUACULTURE ASPECTS OF BAIT RAINBOW TROUT PRODUCTION.** Scott H. Newton and Craig S. Kasper, Cooperative Extension, Virginia State University, Petersburg, VA 23806. Holding and selling of small rainbow trout for live bait to recreational fisheries is a new opportunity for fish farmers in Virginia. Regulations allowing sale of rainbow trout for bait was granted in January, 2001. Two seasons of aquaculture experiments have been conducted to evaluate biological aspects of holding small trout in cages and tanks prior to transportation to bait dealers. The primary condition factor affecting this activity is variable water temperatures. Differences in water temperatures during handling and transportation of small trout is the primary biological challenge of this research. Management of bait trout in cages for live sales during spring appears biologically feasible.

**PHYTONUTRIENTS INTERACTIONS AND POTENTIAL HEALTH BENEFITS: SOY PHYTOCHEMICALS AND PROBIOTICS.** A. Ali<sup>1</sup>, A. Mohamed<sup>1</sup>, S. J. Bhatena<sup>2</sup> & M. T. Velasquez<sup>3</sup>, <sup>1</sup>Department of Biology, VSU, <sup>2</sup>BHNRC/ARS-USDA, Beltsville, MD and <sup>3</sup>George Washington Medical Center, Washington DC. This study was employed to test the hypothesis that the probiotics enhance the absorption of soy isoflavones and increase their biologically active metabolites. Lean and obese SHR/N-cp rats were fed diets containing either 20% casein (control), or 20% casein with either 0.1% isoflavones (I), or 0.1% probiotics (P) containing *Lb. acidophilus* (LA140), *Lb. casei subsp. casei* (LC107) and *Bifidobacterium bifidum* (BBL730), or a mixture 0.1% isoflavones and 0.1% probiotics (IP) for 20 weeks. Plasma was analyzed for metabolic and enzymatic parameters. I diet, with or with out P diet, decreases weight gain and total body fat in both lean and obese rats. I containing diet decreased plasma glucose and cholesterol in lean and obese rats compared to the P and control. Plasma level of creatinine, blood urea nitrogen, uric acid, aspartate aminotransferase and alanine aminotransferase decreased in the lean and obese rats fed I and IP diets. These results indicate that I diets with or with out P diet have differential effects on the plasma parameters and body weight and therefore may be effective in reducing obesity. P diet do not appear to enhance the effect of I diet.

**THE PROSPECTS FOR SWEET CORN IPM IN VIRGINIA.** Mark Kraemer & Carl Niedziela Jr., Agricultural Research and Extension, Virginia State University, Petersburg, VA. Fresh sweet corn is an important summer crop, especially after the establishment of two new farmers markets in eastern Virginia. However, commercial production requires high quality corn ears, at least 90% free of insect or other damage. Corn earworm is the major threat and producers have been known to apply insecticide 3 or more times a week during the 3 week period from first silk to harvest. We tested an IPM approach developed for the northeastern region. Pheromone traps are used to monitor pest populations and time insecticide applications. Each treatment plot consisted of 6 rows of corn, 50 feet long. Treatments were: spray every 3 days, every 5 days, IPM timed, and an unsprayed control. A pyrethroid pesticide, Warrior®, was used. The experiment was repeated over 3 years using 4 or 5 replicates. Corn earworm was the most serious pest, damaging over 90% of the untreated ears. Sap beetles were a primary pest, capable of significant damage. European corn borers were present but not a major problem. Although an IPM monitoring program in southeastern Virginia may reduce

insecticide treatments by 1 or 2 sprays in most years it is questionable whether it is cost effective in Virginia where temperatures are higher and corn earworm populations are greater than in regions farther north.

POTATO LEAFHOPPER (HOPMOTERA: CICADELLIDAE) IN GLANDULAR-HAIRED ALFALFA: PEST DENSITIES, YIELDS, AND FORAGE QUALITY. T. A. Dellinger, R. R. Youngman, & C. A. Laub, Dept. of Entomology, VPI&SU, Blacksburg, VA 24061. Commercial varieties of glandular-haired (GH) alfalfa have been marketed as having resistance to the potato leafhopper (PLH) (*Empoasca fabae* Harris). Pioneer '54H69', a GH cultivar, and Southern States 'Choice', a standard non-GH cultivar, were seeded in Montgomery Co. and Campbell Co., VA, in 1999 to evaluate the role of GH alfalfa within alfalfa pest management. Plots were planted in a randomized complete block design with four combinations of insecticide-treated and untreated '54H69' and 'Choice' cultivars. Treated plots received insecticide application for PLH regardless of pest pressure in order to approximate calendar spraying conducted by growers. Overall, '54H69' did not appear to offer any distinct advantages over 'Choice'. Variety had no effect on PLH densities except at Montgomery Co. in 2000, when significantly fewer nymphs were found in '54H69'. A slight but significantly higher percentage crude protein was found in '54H69' in that year only. Variety had little effect on dry yields. Surprisingly, insecticide application reduced pest pressure but did not necessarily improve yields or forage quality.

CURRENT RESEARCH ON SOYBEAN: OBESITY AND DIABETES. A. I. Mohamed<sup>1</sup>, S. J. Bhathena<sup>2</sup>, A. A. Ali<sup>1</sup>, & M. T. Velasquez<sup>3</sup>, <sup>1</sup>Department of Biology, VSU, <sup>2</sup>BHNRC/ARS-USDA, Beltsville, MD and <sup>3</sup>George Washington Medical Center, Washington DC. Soybean (S) have been reported to have beneficial effects on chronic diseases, including cancer, cardiovascular disorders, and renal disease. We tested the hypothesis that S also has beneficial effect on obesity and diabetes. Lean and obese SHR/N-cp rats were fed diets containing either 20% casein, or 20% S for 26 weeks. Lean rats were hypertensive while obese rats show genetic characteristics of type II diabetes. Obese rats had significantly higher plasma glucose (G), triglyceride (TG), total cholesterol (C), HDL cholesterol (HDL-C) and LDL cholesterol (LDL-C). S significantly decreased C and LDL-C in both lean and obese rats. It had no significant effect on G. S had varying effects on tissue weights in lean and obese rats. Obese rats compared to lean rats, had significantly lower plasma creatine but higher total bilirubin (BIT), blood urea nitrogen, alanine aminotransferase (AT) and lactate dehydrogenase (LDH). S diet decreased BIT, AT, protein and uric acid in lean rats, but the effects in obese rats were mixed. In conclusion, S diet has beneficial effects on metabolic and enzymatic parameters and may play a role in reducing complications of obesity.

EFFECT OF CALCIUM NITRATE CONCENTRATION ON THE GROWTH AND FLOWERING OF DUTCH IRIS (*Iris hollandica*) IN THE FLOAT SYSTEM. C.D. Mullins<sup>1</sup>, C.E. Niedziela Jr.<sup>1</sup>, T.D. Reed<sup>2</sup>, & A. Atalay<sup>1</sup>, <sup>1</sup>Va. Coop. Ext. & Agric. Res., Va. State Univ., Petersburg, VA 23806 & <sup>2</sup>Southern Piedmont AREC, Va. Polytechnic Inst. & State Univ., Blackstone, VA 23824. Dutch iris 'Ideal' bulbs were planted on 30 Oct. 2001 in a tobacco transplant greenhouse in either 32-cell polystyrene trays or lay-flat bags containing tobacco germination media and harvested as cut flowers. The four treatments were lay-flat bags irrigated weekly with a Ca(NO<sub>3</sub>)<sub>2</sub> solution at 400 mg N·L<sup>-1</sup> and polystyrene trays floated on Ca(NO<sub>3</sub>)<sub>2</sub> solutions maintained at 50, 100, and 200 mg N·L<sup>-1</sup>. Treatments were randomized in a Latin-square design with four replications of each system and means separated by least significance difference. Days to first harvest were shorter in lay-flat bags (47 d) compared to float treatments (50 d). Harvest duration was shorter in lay-flat bags (17 d) than float trays at 50, 100, and 200 mg N·L<sup>-1</sup> (44, 42, and 42 d, respectively). Stems from the lay-flat bags (22.8 g) were heaviest, followed by 50 mg N·L<sup>-1</sup> float trays (21.2 g), then 100 and 200 mg N·L<sup>-1</sup> float trays (19.8 g). Stems in the 200 mg N·L<sup>-1</sup> float trays (72 cm) were longer than the lay-flat bags (58 cm). On 4 Dec 2001, there were no significant differences in N, P, K, Mg, and Ca in shoot tissue.

DETECTING *STREPTOCOCCUS* IN TILAPIA FROM COMMERCIAL AQUACULTURE SYSTEMS. David Crosby, Cooperative Extension, PO Box 9081, VSU, Petersburg, VA 23806. *Streptococcus* is an emerging pathogen of tilapia in tanks utilizing recirculating aquaculture systems (RAS) technology. The fish losses caused by *Streptococcus* worldwide are estimated at \$150 million. Recent US surveys have shown that the prevalence of *Streptococcus* in tilapia is about 4 percent. This project looks at detection protocols used for inspecting tilapia for *Streptococcus*. The present inspection protocol for recovering *Streptococcus* from tilapia requires a sixty fish sample with isolates coming from the skin of the fish and inoculated on CNA 5% Blood Agar for 48 hrs at 35° C. Since 1996, Virginia State University (VSU) Fish Health Diagnostic Laboratory has not recovered *Streptococcus* during an inspection of tilapia. However, VSU Fish Health Lab has recovered *Streptococcus* from tilapia exhibiting mortalities in tanks: one case in 2000 and two cases in 2001. As part of the inspection protocols, a routine check is conducted for external parasites. *Ichthyobodo* was the most prevalent protozoan parasite found on tilapia. The prevalence of *Ichthyobodo* on tilapia ranged from 25% to 57%.

PRELIMINARY RESULTS FROM THE USE OF HYDROGEN PEROXIDE IN RECIRCULATING AQUACULTURE SYSTEMS WITH SUMMER FLOUNDER (*Paralichthys dentatus*). David E. Mowry, M.H. Schwarz, R.W. Cool and M.L. Jahncke. Virginia Seafood Agricultural Research and Extension Center, 102 S. King Street, Hampton, VA 23669. Hydrogen peroxide ( $H_2O_2$ ) is a compound used for therapeutic control of external diseases of cultured fish. Considered a low regulatory priority by the U.S. Food and Drug Administration (FDA), many other uses of hydrogen peroxide, in aquaculture, are being developed. Hydrogen peroxide also acts as an oxidizing agent, removing dissolved organics from the water column. Research conducted at the Virginia Seafood Agricultural Research and Extension Center (VSAREC) compared the efficacy of hydrogen peroxide versus protein skimming in recirculating aquaculture systems. Six, 480 gallon independent replicated systems, three with  $H_2O_2$  and three with protein skimmers, were stocked with twenty-nine summer flounder averaging 480 grams/fish. Hydrogen peroxide was added, via IV drip, based on organic loading of feed input. Total suspended solids and bacterial counts were sampled weekly. Weight gain was assessed every three weeks. Preliminary assumptions suggest that hydrogen peroxide was less effective than protein skimming.

CLINICAL PRESENTATIONS OF *MYCOBACTERIUM* SP. IN SUMMER FLOUNDER (*PARALICHTHYS DENTATUS*) HELD IN RECIRCULATING AQUACULTURE SYSTEMS. Kathleen P. Hughes and Stephen A. Smith, Aquatic Medicine Laboratory, Dept. of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, VPI&SU, Blacksburg, VA, 24061. A population of 1000 commercially-reared juvenile summer flounder was housed in recirculating systems for approximately one year. After six months, fish began to develop oral masses on the lower mandible. These discrete masses were generally white-yellow in color and encompassed the rostral portion of the mandible. In addition, fish developed head swelling, exophthalmia, coelomic distention and opercular masses. It was estimated that at least 40% of the entire population displayed one or more of these clinical signs. Impression smears and histopathology of these lesions (stained with Ziehl Neelsen acid-fast stain) revealed a dense population of acid-fast bacilli. All affected tissues had marked effacing and coalescing granulomatous inflammation primarily composed of epithelioid macrophages. This tissue reaction was not the typical teleost granulomata response to *Mycobacterium* sp. Bacterial cultures from the affected tissues grew on Lowenstein-Jensen and Middlebrook media and were confirmed to be acid-fast positive with Ziehl Neelsen staining.

### Astronomy, Mathematics and Physics

PHOSPHONATE-BASED MONOLAYERS ON GaAs AND ITO. Karen Bland, Sharon E. Koh, & Chris Hughes, Dept. of Physics, James Madison University, Harrisonburg, VA 22807. We have demonstrated the formation of self-assembled monolayers (SAMs) of organo-phosphonates on GaAs (100) and (Indium, Tin) Oxide (ITO) surfaces. Monolayers of Octadecylphosphonic acid (ODPA) were formed by immersion of the materials in a millimolar solution for more than 20 minutes. These presence of the monolayer was confirmed by both X-ray Photoelectron Spectroscopy and contact angle measurements. Water contact angles of over  $110^\circ$  confirm nearly complete coverage of the surface by the monolayer. The contact angle does not change significantly over a period of several months of air exposure indicating that these monolayers are very robust. We also demonstrated the modification of the electronic properties of the ITO surface by phosphate monolayers by measuring the work function by photoelectron spectroscopy and Kelvin probe.

CONSTRUCTING WEB-BASED SCIENCE SIMULATIONS. Richard L. Bowman, Dept. of Physics, Bridgewater Col., Bridgewater, VA 22812. Since 1997 the web site, "Interactive Science Activities on the Web" (ISAW), has been under development as a home for simulations relating to physics and astronomy. The goal has been to present simulations that are visualizations of concepts that are not easily illustrated by in-class demonstrations or laboratory experiments, that are interactive so as to enhance student learning, and that are web-based and thus essentially independent of the type of computer used or the browser invoked to run the simulations. With this last criteria in mind, the simulations are written in standard HTML and JavaScript. Web forms and cookies are used to accept values of data chosen by the user which can then be passed to the code in the next-generated web page. A new simulation, "Finding Exoplanets," has recently been added to the site. The two previously active simulations ("Interactive Investigation of the Shapes of Planetary Orbits" and "Interactive Fractal Generation Using Iterative Function Systems") have also been dramatically revised. Each simulation has an associated on-line exercise to guide students in learning from the simulation. ISAW is available on the Internet at:  
<http://www.bridgewater.edu/departments/physics/ISAW/>

PROPAGATION CHARACTERISTICS OF MAXWELL'S EQUATIONS. A. Martin Buoncristiani, Dept. of Physics, Computer Science and Engineering, Christopher Newport Univ., Newport News, VA 23606. In studying the propagation of light through the non-linear optical material KTP (potassium titanyl phosphate, an orthorhombic crystal with unit cell dimensions in the ratios 1:1.66:2.00) I discovered anomalies in the standard numerical algorithm for the direct solution to Maxwell's equations (ME) due to Yee. These anomalies result from the assumption, frequently made in this type of numerical analysis, that ME are over specified and hence it is possible to use only the six curl equations to determine the six components of the propagating electric and magnetic fields. The remaining two divergence equations can either be ignored or used after the computation to check on the consistency of the numerical solution. It is possible to give a rigorous (but not particularly intuitive) proof that the assumption that Maxwell's equations are over specified is not correct. I want to present here a simple constructive proof of this for the case of propagation in a vacuum. This construction delineates the different polarization states of the propagating fields and it suggests a basis for a new algorithm. Generalization of these results to the case of propagation in general matter is described and an improved algorithm for the numerical solution of ME in complex media is given.

GENERATION OF PRIME CHAINS HAVING ACCELERATED GROWTH IN NUMBER OF DIGITS. Charlie H. Cooke, Department of Mathematics and Statistics, Old Dominion Univ., Norfolk, VA 23529. Available numerical evidence shows that the longer chains of Cunningham primes have the last digit of each element fixed. It is shown how to generate prime chains that have this fixed digit character but which have a more rapid growth in the number of digits per element per iterative step. Computer implementation is limited by the necessity of memory chaining.

ANALYSIS OF SURFACE AND ELECTRICAL PROPERTIES OF BONDER TIP MATERIALS. Zachary R. Kostura and Gerald R. Taylor, MSC 7702, Department of Physics, James Madison University, Harrisonburg, VA 22807. The production of microelectronic components uses bonding techniques in which an electric current passes through a resistive metallic tip to increase its temperature. The cyclical nature of this process causes tip wear that affects the quality of the electrical junctions produced. This paper presents the results of an investigation of the correlation between changing surface and electrical properties of bonder tip materials and the number of electro-mechanical cycles the tip material has undergone. Temperature variations in the transient electro-mechanical bonding process were observed using infrared techniques. Surface properties of tip material at specific cycles were determined using various microscopy techniques, including optical observations and scanning electron microscopy. Electrical properties at different position on the bonder tips were measured using four-probe resistance techniques. The results of this investigation and the inherent effects of these changing materials properties on the manufacturing environment in the production of microelectronic junctions are discussed. This research was supported by a Virginia Academy of Science Undergraduate Research Grant and by the JMU Center for Materials Science.

FIRE, ICE, WATER, AND DIRT: A SIMPLE CLIMATE MODEL. John Kroll, Dept. of Mathematics and Statistics, Old Dominion University, Norfolk, VA. 23529. The purpose was to develop a simple climate model as a modeling exercise. The spirit was to add a few more simple concepts to combine the simplicity of a toy climate model such as that of Posmetair with the concept of "Daisy World" of Sanders. No really expert knowledge of climatology was invoked, but rather elementary concepts of physics of heat were used. Though the model is crude, the results are surprisingly realistic.

TECHNIQUES: THE ANALYSIS OF PHOTOMULTIPLIER TUBES. Jason C. Mace & Kevin L. Giovanetti James Madison University. Photomultiplier tubes are used in a wide range of applications. Ever since scintillation detectors became commonplace in particle physics research, photomultiplier tubes have been devices that physicists have tried various ways to characterize. In this talk a brief overview of James Madison's detector characterization laboratory will be given along with an introduction to photomultiplier tubes. Presentation will be made concerning the crucial features of photomultiplier tubes in a timing experiment. Furthermore results of TDC and ADC measurements of the single photon peak will be discussed as a way to analyze the intrinsic time of photomultiplier tubes. Future measurements and experimental design techniques will also be presented.

IMPLEMENTING CELESTIAL MECHANICS IN LAHEY FORTRAN. Charles E. Martin & Joseph W. Rudmin, Dept. of Physics, James Madison Univ. Harrisonburg, VA 22807. A brief introduction to the theory and usage of the Parker-Sochacki method of numerically solving systems of differential equations is presented. An example is offered by way of the method's application to solving the differential equations that model the motion of a large amplitude pendulum. In addition, the relevance of the method to solving the differential equations of motion for the planets of the solar system is discussed, along with an overview of a Lahey Fortran program that implements the results. Emphasize is placed on the computational power that results from combining numerical algorithms derived from the Parker-Sochacki method with the computational speed of modern computers.

CORRELATIONS BETWEEN POLLING SCORES, AP EXPERIENCES AND OVERALL GRADES IN INTRODUCTORY PHYSICS COURSES. William W. McNairy, Dept. of Physics, Duke University, Box 90305, Durham, NC 27708-0305 (mcnairy@phy.duke.edu). Recent data obtained from introductory Physics courses (one for Life Sciences majors and one for Engineers) reveal interesting correlations between polling scores, AP experiences and the overall grades earned in the courses. In the lecture part of these courses each student employs an infrared polling device (Personal Response Systems [PRS]) to respond to questions asked by the lecturer. Conceptual and

computational questions are presented that relate to reading assignments, to examples worked in lecture and to demonstrations that have been (or will be) done for the students. Some questions are repolled without display of the initial distribution of answers in order to encourage 'peer instruction' between the students. PRS scores were based upon the frequency, not the correctness, of the responses. Analysis of this data reveals interesting differences between the two student populations. I will also present correlations between prior physics coursework done in high school (as demonstrated by AP level exams on either the B or C levels), the PRS scores, and the overall grades. Dr. Lawrence Evans of this department has provided invaluable support in the development of this analysis.

DEVELOPMENT OF A BRIDGMAN CRYSTAL GROWTH FACILITY FOR II-VI SEMICONDUCTORS. O. Poku, Ei Ei Nyein, A.G. Bluiett, & U. Hommerich, Hampton University, Department of Physics, Hampton, VA 23668. The development of mid-infrared (MIR) laser sources is of great current interest for applications in laser remote sensing and medicine. The newest class of MIR solid-state lasers is based on  $\text{Cr}^{2+}$  doped II-VI semiconductors. Jointly with Brimrose Corporation of America, researchers at Hampton University have demonstrated lasing around 2500 nm from Cr: CdMnTe and Cr: CdTe. In an effort to optimize these materials and to explore other Cr based laser crystals, a new Bridgman crystal growth facility has been developed at Hampton University. Three Bridgman stations with computer-controlled translation mechanisms are currently operational. Each crystal growth station uses a three zone-furnace in order to fine adjust the furnace profile for optimization of the crystal growth process. The first successful growth of undoped and Cr doped CdTe materials will be described in this presentation.

ANALYSIS OF YEAR LONG ELF DATA WITH A DISCUSSION OF SOURCES, PERIODS AND LARGE SINGLE EVENTS. Michael Wallace & John Wallace, Hampden-Sydney College and Casting Analysis Corporation. The ELF Spectrum (1-1230 Hz) is a turbulent region of the electromagnetic spectrum. Geomagnetic Storms and UV radiation cause the ionosphere's properties to change drastically over time. With a narrow band, low noise receiver and two induction coils oriented in the cardinal, magnetic directions, magnetic field data was obtained at fourteen frequencies for ten months. The data shows at low frequencies geomagnetic storm polarization favors the east-west direction more than the north south. In the Fourier transform of the data, Periods greater than one day modulate the daily period raising the amplitudes around the daily peak above zero. A characteristic  $1/f^2$  fall of in the amplitude occurs due to simultaneous variations in both the conductivity and electric fields of the ionosphere of which both properties have  $1/f$  dependencies. After plotting a spectrum with periodic data, a maximum at two hundred hertz appears which is a lower cut-off in the band.

PARTICLE ACCELERATOR CALIBRATION SYSTEMS. Andrew W. Werner & Kevin L. Giovanetti, Department of Physics, James Madison University, Harrisonburg, Va. 22807. The  $\mu\text{Lan}$  collaboration is an effort to determine the lifetime of the muon to one part per million. This will be a significant improvement over the current accepted lifetime of about 60 parts per million. In July the collaboration has about a month of muon beam time in Switzerland. For the data collected in the detector to make sense we first need a working, reliable calibration system. The detector is shaped like a soccer ball, comprised of pentagons and hexagons which are in turn comprised of five or six triangles. For each triangle there are two sets of scintillators, light guides, and photomultiplier tubes. The scintillators absorb in the UV (approx. 350 nm) and emit blue photons (approx. 410 nm). In the calibration system we emulate actual conditions with light emitting diodes (LEDs). Also of concern is the pulsing of these LEDs in order to have them emulate experiment situations as well. For this we need a pulse with only a few nanosecond width that can be run at frequencies of about 15 kHz. We are using a modified version of the KamLAND pulser created by their calibration group. With our modifications these pulsers will create the reliable yet versatile calibration system that this experiment depends on.

THE EFFECT OF PERCENTAGE OF SOLID CONTAMINANT IN A LIQUID SOLUTION ON THE VELOCITY AT WHICH THE SOLUTION PASSES THROUGH A SINGLE-LEAF FILTER SYSTEM. Xun Zhou, Manchester Middle School, Richmond, Va. 23235. Corrosion of the filter leaf is the main cause of the degradation of filters. The effect of this build up of contaminant particles on the velocity of the fluid traveling the filter is apparent in the function of fluid velocity vs. time (an inverse bell curve). This experiment was conducted to find a method of measuring the amount of contamination in a filter using the velocity of the fluid passing through the filter. It was hypothesized that if distilled water was sent through a GE SmartWater<sup>®</sup> Filter in which the percentage of granular sand contamination was altered (0%, 5%, 10%, 15%, 20%), the rate of change could be expressed algebraically, along the lines of  $\Delta V = N/C$  (the velocity of the water is proportional to the number of pores in the filter over the percentage of contamination). The hypothesis was partially correct (the actual graph was similar to the hypothesis), but not completely. A more suitable equation is  $\Delta V = -0.1162C + 0.5275$ . The difference from the hypothesis was due to the interference caused by other constants and variables that were unaccounted for. Further experimentation could be conducted to determine these other factor's attributes.

### Biology

THE EFFECTS OF AZASERINE ON THE P53 GENE IN BREAST CANCER CELLS CRL-2314. Monica A. Bintz & Rosemary Barra. Dept. of Biol. Sci., Mary Washington College, Fredericksburg, VA 22401. Cytotoxic amino acid analogues including azaserine, an antagonist of L-glutamine, have shown anti-tumor activity in a number of studies. *In vitro*, this antimetabolite is a potent inhibitor of several glutamine-dependent aminotransferases, resulting in inhibition of *de novo* purine biosynthesis. In addition, azaserine acts as a carboxymethylating agent producing N7-carboxymethylguanine-DNA and O<sup>6</sup>-carboxymethylguanine-DNA. The tumor suppressor gene, p53, is often referred to as the guardian of the cell. It can trigger mutated cells to stop their progression through the cell cycle, giving the cell an opportunity to repair the DNA damage. When the cell is unable to repair the damage, p53 will trigger apoptosis. The purpose of this study was to determine the cytotoxic activity of azaserine against CRL-2314 breast adenocarcinoma cells and to determine the effects of the drug on the expression of the p53 gene. The MTT cytotoxicity assay indicated that a 24 hour incubation with 1µg/ml of azaserine reduced cell viability 72% and immunoblot analysis indicated an increased expression of p53. These results suggest that DNA lesions may be involved in the cytotoxic activity of azaserine in CRL-2314 cells.

STUDY OF TELOMERASE PROMOTER REGION IN A PROSTATE MODEL SYSTEM. Patrick C. Sachs<sup>1</sup>, Shawn E. Holt<sup>2</sup>, Keith O. Jenson<sup>2</sup>, <sup>1</sup>Department of Biology, Va. Commonwealth Univ., <sup>2</sup>Department of Pathology, Medical College of Va., Va. Commonwealth Univ. The extension of telomeres by telomerase causes cell lines to bypass senescence and allows the cells to proliferate indefinitely. The prostate cancer cell line P69 was used with two variations of the parental P69 and P69 itself. The two variations where P69 hTERT, which has been infected with the catalytic subunit of telomerase hTERT, and P69 pBABE, which is the control for the hTERT infected P69 with only the retroviral vector pBABE puro inserted. The cells where allowed to grow until approximately 60-80% confluent in modified RPMI media. The three cell types where then transfected with three different vectors, a positive control using the sv40 promoter enhancer and a luciferase gene, a negative control with nothing but the vector and the same vector with only the promoter region of hTERT. After transfection the cells where allowed to grow for three days and then the cells where passively lysed and a luciferase assay was performed. The results showed an increase of approximately two-fold promoter region activity in the P69 hTERT infected cells over the P69 parental and P69 pBABE. This could indicate that the transcription factor for hTERT is up regulated with the addition of more hTERT.



CDNA MICROARRAY ANALYSIS IN HYPO- AND HYPERCALCEMIA MODELS OF RENAL GENE REGULATION AND ASSOCIATION WITH DISEASE AND AGING. Amandeep Bajwa, M. J. Beckman, Department of Biochemistry, VCU, Richmond, VA 23298. The active form of vitamin D-1,25 dihydroxyvitamin D<sub>3</sub> (1,25-(OH)<sub>2</sub>D<sub>3</sub>) is converted from 25-hydroxyvitamin D<sub>3</sub> mainly in the kidney proximal tubules by the enzyme 1 $\alpha$ -hydroxylase (1 $\alpha$ -OHase). The synthesis of 1,25-(OH)<sub>2</sub>D<sub>3</sub> is regulated by dietary Ca<sup>2+</sup>, by Parathyroid Hormone (PTH) to increase 1 $\alpha$ -OHase, and by 1,25-(OH)<sub>2</sub>D<sub>3</sub> which regulates its own synthesis through a 1,25 dihydroxyvitamin D<sub>3</sub>-receptor (VDR)-dependent negative feedback effect on both 1 $\alpha$ -OHase and PTH. Rats were fed diets containing 0.02% calcium (-Ca<sup>2+</sup>) or 0.47% calcium (+Ca<sup>2+</sup>). Some of the -Ca<sup>2+</sup> and +Ca<sup>2+</sup> rats were given daily oral doses of different levels of vitamin D<sub>3</sub>, 0 (-D), or 4 $\mu$ g D<sub>3</sub>/day, (+D), also the +Ca,+D group was treated with 1,25-(OH)<sub>2</sub>D<sub>3</sub>. Using oligonucleotide microarray analysis, we screened hypo- and hypercalcemic sets of RNA to further study factors that down-regulate VDR and thereby may prove important as therapeutic targets of aging or disease. Ca<sup>2+</sup> and PTH receptors were both increased in hypo- compared to hypercalcemia. In addition, numerous gene expression products not previously linked to VDR regulation were screened and identified in both RNA sets.

RELATIONSHIP BETWEEN P53 AND P21 IN ME180 CELLS. Tracy Brandt & Rosemary Barra, Dept. of Biol. Sci., Mary Washington College, Fredericksburg, VA 22401. Human papillomavirus-infected cervical carcinoma cells express the E6 oncoprotein that binds to wild-type cell cycle control-protein TP53 (p53). This binding targets TP53 for early degradation and this action is linked to the development of the neoplasia. However, some of the cellular TP53 does not come in contact with oncoproteins and remains intact for its normal lifespan. ME-180 cervical carcinoma cells were treated with Calbiochem p53 Activator Fusion Peptide 46. This cell-permeable protein represents the activated ssDNA-binding end of TP53 and it releases the sequence-specific DNA binding activity of the TP53 core from its negative control. As a result, this treatment induced the ME-180 cells to develop senescent, and possibly apoptotic, characteristics. Cells also underwent TP53-dependent apoptosis when exposed to etoposide, confirming that this pathway is intact. The induction of senescence and apoptosis was observed using light microscopy, TUNEL assays, and a  $\beta$ -galactosidase assay. Therefore, it may be concluded that activation of the remaining TP53 is sufficient to induce senescence or apoptosis and should be investigated further as part of a potential treatment for this type of cancer.

NICOTINE AND HYPOXIA INDUCED APOPTOSIS IN H9C2 CARDIOMYOBlasts. Daryl W. Williams & Rosemary Barra, Dept. of Biol. Sci., Mary Washington College, Fredericksburg, VA 22401. Apoptosis is an energy-dependent, programmed form of cellular death that can be triggered by a variety of intrinsic and extrinsic factors. Although cells contain a number of apoptotic signaling pathways, these pathways converge on a final common death sequence that is activated by a family of cysteine proteases, *caspases*. Occurrence of apoptosis and the behavior of BCL-2 and caspase-8 were examined in H9c2 cardiomyoblasts in response to exposure to nicotine and hypoxia. Cells exposed to hypoxia for 6 hours followed by reoxygenation for 2, 4 and 6 hours demonstrated 125%, 7.4% and 28.6% increases respectively in caspase-8 activity. Cells exposed to 0.5 mg/ml nicotine for identical periods of time also showed increases in caspase-8 activity, 3.2%, 5.1% and 42.8% respectively. These cells demonstrated DNA laddering on agarose gels and floating cells in cultures treated with 0.5 mg/ml nicotine for two hours demonstrated nuclear condensation as detected via propidium iodide staining and fluorescent microscopy. Adherent cells in these cultures showed a 343% increase in caspase-8 activity. These data suggest that H9c2 cardiomyoblasts undergo apoptosis via a caspase 8 dependent pathway when exposed to nicotine or hypoxia followed by reoxygenation.

MITOCHONDRIAL DYSFUNCTION IN NEURODEGENERATION: APOPTOSIS, CYTOSKELETAL DISRUPTION AND TRANSPORT ABNORMALITY. Kathleen J. S. Griffioen<sup>1</sup>, Othman Ghribi<sup>2</sup>, Nena Fox<sup>3</sup>, Michael S. Forbes<sup>2</sup>, Robert S. Brindle<sup>1</sup>, John Savory<sup>2</sup>, & David A. DeWitt<sup>1</sup>. <sup>1</sup>Dept. Biology and Chemistry, Liberty University, Lynchburg, VA 24502, Dept. Pathology and Dept. Microbiology, University of Virginia, Charlottesville, VA. Previously, we developed an *in vitro* model of neurodegeneration utilizing aluminum maltolate (Al) to trigger the death of human neuroblastoma cells (NT2). Electron microscopy was used to verify nuclear fragmentation consistent with an apoptotic morphology. Al treated cells were TUNEL positive and confocal microscopy demonstrated cytochrome c release in some cells. Surprisingly, mitochondria clustered in the peri-nuclear region suggesting a disruption of intracellular transport. In addition,  $\beta$ -tubulin immunocytochemistry demonstrated an accumulation of this protein also in the peri-nuclear region. In differentiated cells, long neuronal processes lacked neurofilament protein and had few mitochondria. We also observed swollen regions in these processes with clumps of mitochondria and cytoskeletal proteins. Taken together, these results suggest apoptosis and cytoskeletal changes may be linked. Supported by the Jeffress Memorial Trust J-572, and the Virginia Academy of Science (KJSG).

INHIBITION OF LIPOPOLYSACCHARIDE-INDUCED PREGNANCY LOSS IN CD-1 MICE BY TREATMENT WITH HEPARIN AND ASPIRIN. Alison M. Warren<sup>1</sup>, Carolyn M. Conway<sup>2</sup>, and Arthur F. Conway<sup>1</sup>. <sup>1</sup>Dept. of Biol., Randolph-Macon Coll., and <sup>2</sup>Dept. of Biol., Virginia Commonwealth Univ. Lipopolysaccharide (LPS) from the outer cell wall of Gram-negative bacteria causes pregnancy loss in mice. Ten mice received low dose heparin (3.8 units/day) injected subcutaneously (s.c.) plus aspirin (31  $\mu$ g/day in 0.095% ethanol as drinking water), 10 mice received higher dose heparin (7.6 units/day s.c.) plus aspirin (62  $\mu$ g/day in 0.095% ethanol as drinking water), and 10 control mice received PBS injections (s.c.) and drinking water with 0.095% ethanol on days 8-11 of gestation. On day 9, 5 mice from each group received LPS (5  $\mu$ g in 0.05 ml PBS i.v.) and the other 5 mice from each group were injected with PBS. All mice were sacrificed on day 12. The frequency of pregnancy loss was significantly increased by LPS treatment. The lower dose of heparin and aspirin reduced the frequency of pregnancy loss in 3 of the 5 LPS-injected females, but the difference was not statistically significant. The higher dose of heparin and aspirin had no effect on LPS-induced pregnancy loss. These results suggest that heparin and aspirin may be able to inhibit LPS-induced pregnancy loss in mice, but were not consistently effective at the doses used in this study.

GENE TRANSFER IN *Porphyromonas gingivalis*. Jovonni R. Spinner & Kevin Jones, Dept. of Biology & Philips Institute, Oral Molecular Biology, Virginia Commonwealth University, Richmond, VA 23284. *Porphyromonas gingivalis* is an anaerobe that is a member of the black-pigmented group Bacteroides. It is the major contributing factor in periodontal disease. Currently, a gene reporting system does not exist for *P. gingivalis*. Two different mutant strains of *P. gingivalis*, FLL 100 (tetracycline resistant) and W83 (erythromycin resistant), were mated via conjugation with each other or with *Bacteroides fragilis* (rifamycin resistant) to determine if gene transfer was possible between the different strains. Several matings were set up using different ratios because it was not known which strain would be the donor or recipient. Based on the results, it was concluded that gene transfer did not occur. In addition, strains of *Porphyromonas asaccharolytica* (2256 and 1912) and *P. gingivalis* (YH and F2) were screened for plasmid DNA using the Qiagen mini prep kit and large scale Cesium preps. Based on the results from the gels, plasmids were detected in *P. asaccharolytica* but no plasmids were detected in *P. gingivalis*.

THE ISOLATION AND STUDY OF HALOPHILIC ARCHAEA FROM THE SALARS OF THE ATACAMA DESERT IN CHILE. J. Harris Carpenter & Carol D. Litchfield, Dept. of Biol., George Mason University, Fairfax, VA 22030. The Atacama Desert of Chile can be considered a hyper-arid and extreme environment. Salars of the desert have been found to contain halophilic microorganisms. Using PCR and amplicon length heterogeneity (ALH) fingerprinting, a salt sample from the desert

was found to contain various strains of halophilic Archaea. The isolated cultures were determined to grow most efficiently on Modified Casamino Acid medium with Tap water (MCAT) containing 15% solar salt and incubated at 37°C. Different sources of carbon and amino acids than those normally used in MCAT were explored. Organisms that grew on these new variations were not transferable back to standard MCAT. None of these organisms would grow in standard selective media. Future methods for identification of the isolated cultures include inoculation of API NE 20 selective media strips, BIOLOG metabolic tests, and 16S rRNA sequencing.

**BOTTLENOSE DOLPHIN UTILIZATION OF THE ELIZABETH RIVER, VIRGINIA.** Kevin M. Foss & James R. Reed, Dept. of Biology, Chemistry and Environmental Science, Christopher Newport University, Newport News, VA 23606. As the Elizabeth River is both toxin laden and extensively used by commercial, military and pleasure craft, it presents a unique and previously unstudied habitat for Bottlenose dolphins (*Tursiops truncatus*). Using standard protocols for dolphin research, 43 cruises were made over the course of three years and data was recorded on behavior, location and number and animals were recorded on film. The most common behavior observed was feeding. An annual pattern of usage of the river began in May, with a peak in August and no activity from November to April. A diurnal pattern of movement appeared to be used, with no effects seen due to tidal state or weather. Spatially, the dolphins used the main branch of the river most frequently, but with common use of the Lafayette River, a tributary. Using the photo ID data, 125 individuals have been identified using the Elizabeth River during the summer of 2000. A Caughley recapture estimate was used to derive a total population estimate of 216. Habituation to small boat traffic has been noted, as has reactions such as bunching and extended diving near larger vessels. A solitary dolphin overwintering for the past two winters and seen begging has been found dead from propeller strike.

**A SURVEY OF THE BATS OF A. P. HILL, CAROLINE COUNTY, VIRGINIA.** A. Scott Bellows<sup>1</sup> & Joseph C. Mitchell<sup>2</sup>, <sup>1</sup>Dept. of Biol. Sciences, Old Dominion University, Norfolk, VA 23529 & <sup>2</sup>Dept. of Biol., University of Richmond, Richmond, VA 23173. We monitored bat activity on Fort A.P. Hill, Caroline County, Virginia, using mist nets (72 nights) and ANABAT® systems (24 nights) during Apr-Oct 2000 and Apr-Aug 2001. A total of 40 sites were sampled that were classified into three forest (hardwoods [HW], mixed pines and hardwoods [MX], pines [PN]) and three habitat types (permanent waters [PW], riparian corridors [RC], uplands [UP]). We captured 407 bats in mist nets, representing eight species: *Lasiurus borealis* (n=281), *Eptesicus fuscus* (47), *Pipistrellus subflavus* (36), *Myotis septentrionalis* (13), *M. lucifugus* (12), *Lasionycteris noctivagans* (9), *L. cinereus* (3), and *Nycticeius humeralis* (5). There were no differences ( $F=0.64$ ,  $P=0.535$ ) in overall captures/100 net nights (NN) among forest types. Overall captures/100 net nights (NN) differed ( $F=3.31$ ,  $P=0.047$ ) among habitat types; post-hoc analyses could not tease out significantly different pairs. Captures/100NN for *L. borealis* were higher ( $F=3.93$ ,  $P=0.028$ ) for PW than for RC and UP. No other significant differences in captures of species among habitat types or forest types were revealed. The ANABAT® system detected a higher number of bat spp./night ( $t=1.17$ ,  $P<0.00001$ ) than mist nets.

**MALE SONG REPERTOIRE SIZE AND FEMALE CHOICE IN THE GRAY CATBIRD.** C. E. Clarkson, E. T. Haas, J. K. Miller, & A. S. Dolby, Department Biological Sciences, Mary Washington College, Fredericksburg, VA 22401. Sexual selection theory proposes that elaborate male secondary sexual characteristics have evolved in some species because they confer a mating advantage upon their bearers. Such characters may make males more attractive to females by reflecting their genetic quality and/or their ability to provide females with direct reproductive benefits. The Gray Catbird is sexually monomorphic in appearance. However, males sing elaborate songs during the breeding season while females sing little. We tested the hypothesis that female choice for large repertoire size is a selective pressure that has produced song sexual dimorphism in catbirds. We indexed song repertoire size by counting the number of unique phrase types produced by each male during a five-

minute sample of song recorded in separate one-minute bouts. We found no correlation between song repertoire size and either pairing date or any of the parameters of male physical condition that we measured. We did, however, find a suggestive relationship between repertoire size and male chick feeding rates relative to their mates'. This study was funded by the Jessie Ball DuPont Foundation Summer Research Program and Mary Washington College.

**BOTTLENOSE DOLPHIN INFANTICIDE IN VIRGINIA.** D.M. Boyd, S.G. Barco & W.M. Swingle, Virginia Marine Science Museum Stranding Program, Virginia Beach, VA 23451 Old Dominion Univ., Norfolk, VA 23529. Examination of stranded bottlenose dolphins (*Tursiops truncatus*) led to the discovery of infanticide in Virginia in 1997. For this study, we examined post 1997 *Tursiops* calves (<200cm) from Virginia for evidence of traumatic injuries associated with infanticide. Using <160cm total length as a crude definition of young of the year (YOY), we compared YOY stranding data from Virginia with YOY strandings in other mid-Atlantic states (NJ-NC). The number (n=49) and percent (36%) of stranded *Tursiops* YOY were significantly higher (1 Factor model I ANOVA;  $F=8.63$ ,  $df$  6 and 27,  $p<0.001$ ) at the 36° of latitude (which includes all of Virginia beach) than all other latitudes examined (40°-33°). Ten traumatized YOY from 1998-2001 in Virginia were fresh or moderately decomposed and had multiple broken bones, soft tissue damage and bilateral injuries consistent with those examined in 1997, but had no significant gender difference as was observed in 1997. Eight of the ten traumatized YOY had fetal folds or lines and 70% had four or more neonatal characteristics. None of the traumatized YOY showed evidence of fishery interaction.

**REPRODUCTIVE CORRELATES OF AN ANAL GLAND IN THE HISPID COTTON RAT.** Robert K. Rose, Department of Biological Sciences, Old Dominion University, Norfolk, Virginia 23529-0266. During a study of the annual cycle of reproduction in the hispid cotton rat (*Sigmodon hispidus*) in southeastern Virginia, my students and I discovered an anal gland that is present only in males. The anal gland rings the lower end of the rectum and has ductal connections to the urethra, through which its secretions likely are delivered. This fatty gland is highly developed during the breeding season but, like the testes and accessory glands, regresses during the winter non-breeding season. The cyclicity of the anal gland, which is highly correlated with that of testes and of seminal vesicles, suggests that it somehow facilitates normal reproduction. The combined mass of testes, seminal vesicles, and anal gland constitutes only about 0.1 percent of an adult male's body mass during the winter months, but as much as 4 percent of body mass during the breeding season. Thus, males devote a large amount of energy to growing and maintaining these glands in anticipation of and during the breeding season. The function of the anal gland and the nature of its secretion are unknown.

**VASCULATURE OF THE PAROTOID GLANDS OF *Bufo terrestris* AND *B. valliceps*.** Deborah A. Hutchinson & Alan H. Savitzky, Dept. of Biol. Sci., Old Dominion University, Norfolk, VA 23529. The parotoid glands of toads are large aggregations of granular glands located above the shoulders. To determine the vascular pattern of these glands in *Bufo terrestris* and *B. valliceps*, we perfused the vascular system of specimens with either India ink or Microfil, a fine latex. The perfused glands were then examined by gross dissections, microscopic examinations, and histology. The parotoid glands of both species are supplied with blood by the dorsal ramus of the cutaneous artery and drained by a branch of the internal jugular vein. This pattern of blood flow has not been described previously for the parotoid glands and conflicts with prior accounts for other congeners. The internal jugular vein of *B. terrestris* and *B. valliceps* receives both the vertebral vein and a branch from the parotoid gland. The artery that supplies the parotoid gland and the vein that drains it ramify into capillaries that surround the individual lobules of the gland. Extensive vasculature presumably is important for delivering cholesterol and other molecules that are precursors to the toxins synthesized in the parotoid glands.

A COMPARATIVE STUDY OF DERMAL MORPHOLOGY IN BOID SNAKES. Troy Shell<sup>1</sup>, Victor R. Townsend, Jr.<sup>1</sup> & Alan H. Savitzky<sup>2</sup>. <sup>1</sup>Virginia Wesleyan College, 1584 Wesleyan Drive, Norfolk/Virginia Beach, VA 23502 and <sup>2</sup>Department of Biological Sciences, Old Dominion University, Norfolk, VA 23529. Despite the functional significance of the skin of snakes for both feeding and locomotion, relatively few studies have investigated the relationship between adaptation to particular habitats and the structure of skin. In addition, prior studies of skin structure have focused almost entirely upon the epidermis. We examined the dermis of snakes of both subfamilies of Boidae, Boinae and Erycinae. Our sample included arboreal taxa (*Corallus*), fossorial taxa (*Calabaria*, *Eryx*), and species of terrestrial or generalized habits (*Boa*, *Candoia*). For each taxon, samples of skin were removed from anterior, middle, and posterior regions of the body. Skin samples were embedded in paraffin and sectioned with a rotary microtome. Serial transverse and oblique sections were stained with either iron gallein or a trichrome stain to reveal the distribution of elastin or collagen fibers, respectively. Our results indicate that the dermis of boas differs markedly from the condition observed in a more derived group, the colubroids. In addition, we observed intergeneric variation in the density and distribution of elastin fibers.

A COMPARATIVE STUDY OF THE INTEGUMENT OF COLUBRID AND VIPERID SNAKES. Victor R. Townsend, Jr.<sup>1</sup> & Alan H. Savitzky<sup>2</sup>. <sup>1</sup>Virginia Wesleyan College, 1584 Wesleyan Drive, Norfolk/Virginia Beach, VA 23502 and <sup>2</sup>Department of Biological Sciences, Old Dominion University, Norfolk, VA 23529. Relatively little is known about the morphology of the dermis for most species of reptiles. In particular, the structural and mechanical properties of the skin of snakes remain largely unexamined. In this study, we investigated the distribution of elastin and collagen fibers in the dermis of representative species of colubroid snakes. We examined the skin of five species of Colubridae (*Coluber constrictor*, *Dasypeltis scabra*, *Elaphe obsoleta*, *Nerodia fasciata*, *Ophedryx aestivus*), six species of Viperidae (*Agkistrodon contortrix*, *A. piscivorus*, *Atropoides nummifer*, *Calloselasma rhodostoma*, *Crotalus atrox*, *C. horridus*), and one species of Atractaspididae (*Atractaspis bibronii*). For each taxon, serial transverse sections were stained with either iron gallein or a trichrome stain to reveal the organization of elastin or collagen fibers, respectively. Our results indicate that the elastin fibers are largely confined to the stratum compactum, the deeper layer of the dermis. The overlying stratum laxum consists mainly of loosely organized collagen fibers. Considerable interspecific variation in dermal morphology and skin folding of intersquamous skin was observed.

SPECIFICITY OF ORGANIC ANION TRANSPORT IN MALPIGHIAN TUBULES OF THE CRICKET, *ACHETA DOMESTICUS*. R. Kauffman, N. Burkholder, A. Jenner, and D. Graber Neufeld, Dept. of Biology, Eastern Mennonite University. The dye fluorescein (FL) is a substrate of the organic anion transporter (OAT) in mammals. Using epifluorescence microscopy, we characterized the specificity of FL transport in crickets. The OAT inhibitors *p*-aminohippuric acid (PAH, 3 mM) and glutarate (250  $\mu$ M) caused no significant inhibition of FL uptake. As in mammals, a monocarboxylic acid series (3 mM) showed increasing affinity with increased carbon chain length. Two substances totally blocked FL accumulation: probenecid (1.0 mM), an OAT inhibitor, and verapamil (0.1 mM), a substrate of P-glycoprotein. The insecticide chlorpyrifos and its oxon did not cause significant inhibition of FL uptake at 0.5 mM concentrations. The phenoxy herbicide 2,4-D did inhibit FL uptake by 50%. Nicotine, a plant alkaloid, did not inhibit FL accumulation. Our tests demonstrate the presence of a vigorous, multispecific transport system for organic substrates in the Malpighian tubules of crickets. The fluorescein transporter in crickets does have OAT-like characteristics, including monocarboxylic acid affinity and probenecid uptake, however the transporter also displays several novel characteristics such as verapamil transport and low PAH and glutarate affinities.

COMPUTER-ASSISTED ANALYSIS OF COTTON STAINER INSECT VARIATION: PRELIMINARY RESULTS. Harold J. Grau, Dept. of Biol., Chem., & Env. Sci., Christopher Newport Univ., Newport News, VA 23606. Cotton stainers (*Dysdercus* sp.) are pan-tropical hemipterous insects that feed primarily on Malvaceous plants. Several distinct populations of *D. andreae* are found on St. Thomas, USVI. Data previously collected by hand in the field suggested that there may be significant morphological variation among these populations, possibly as a result of geographic isolation. More recently, we have been building a collection of digital images of these insects so that analyses of body size parameters and wing spot patterns could be computerized, using the NIH Image application. Preliminary results indicate that significant differences in body size parameters among populations from various locations do exist.

NOCTURNAL THERMOREGULATION IN THE WHITE-THROATED SPARROW. K. M. Stechler, V.S. Davis, A.S. Dolby, & J.G. Temple. Department of Biological Sciences, Mary Washington College, Fredericksburg, VA 22401. Nocturnal hypothermia has been observed in a number of bird families. However, possible use of this energy-saving strategy by members of family Emberizidae has been little investigated. We used temperature sensitive radio-transmitters to determine whether White-throated Sparrows employ nocturnal hypothermia during winter. We obtained skin temperature measurements on 13 free-ranging sparrows for three consecutive days and nights per subject. The majority of sparrows reduced their body temperatures by 2-3 degrees C, which is not reduction enough to confidently suggest use of hypothermia. A 2-degree drop in body temperature is simply sleep-related in most species. However, three subjects reduced their body temperatures by an average of 5 degrees or more. The maximum reduction recorded for all subjects was 8.5 degrees. Thus, White-throated Sparrows exhibit variability with regard to nocturnal hypothermia use. Preliminary results show no correlation between depth of body temperature reduction and 1) ambient temperature, 2) body mass, and 3) body fat index. We are continuing to investigate the source of this variation. This project was funded by an Undergraduate Research Grant from Mary Washington College.

A COMPARISON OF THE DEFENSIVE BEHAVIORS OF RATSNAKES AND WATERSNAKES. Ryan Killarney & John Temple, Dept. of Biol. Sciences, Mary Washington College, Fredericksburg, VA 22401. Snakes display a variety of defensive behaviors ranging from striking to coiling and death-feigning. We studied the defensive behaviors of juvenile banded watersnakes (*Nerodia fasciata*) and black/yellow ratsnakes (*Elaphe obsoleta*). Snakes were placed in a testing arena, given simulated predatory attacks at five different body regions and the behavioral responses were recorded. Both species consistently displayed the aggressive behaviors of gaping, striking or biting and no species-specific differences were found. Thrashing behavior was consistently displayed by watersnakes in response to stimuli at all body regions and was statistically more prevalent in watersnakes than in ratsnakes for four of the five body regions ( $p < 0.05$ ). Coiling behavior in ratsnakes was suggestively more prevalent than in watersnakes when stimulated at the posterior body regions ( $p = 0.056$ ). These results suggest that aggressive behaviors are important defense mechanisms for both species. Thrashing behavior may be an important escape mechanism for watersnakes, possibly due to their semi-aquatic habitat. This project was funded by an Undergraduate Research Grant from Mary Washington College.

A NOVEL APPROACH IN CLONING, OVER-EXPRESSING AND PURIFYING *E. COLI*'S TRNA GENE AND ITS PRODUCT, TRNA M<sup>5</sup>U54 METHYLTRANSFERASE (RUMT). LaToya Griffin & Cecile Andraos-Selim, Dept. of Biological Sciences, Hampton University, Hampton, VA 23668. More accurate and less laborious techniques are needed for cloning, expressing and purifying bacterial tRNA modification enzymes. There are fifty genes in *E. coli* that are responsible for tRNA modification, one of which is the *trmA* gene that codes for the enzyme, tRNA m<sup>5</sup>U54 methyltransferase (RUMT). This enzyme catalyzes the methylation of uracil at position 54 of the tRNAs' TΨC loop. RUMT increases the fidelity of protein synthesis, influences the rate of protein

synthesis and stabilizes the three dimensional structure of tRNA. Our goal is to produce ample amount of purified *E.coli* RUMT using reproducible, simple and fast techniques. In this research project we have demonstrated that *trmA*, with appropriate primers, is properly amplified using the Polymerase Chain Reaction (PCR). We demonstrated that the amplified PCR product is cloned into a pET expression vector by way of TOPO cloning and purified using immobilized metal affinity chromatography. Future implementations of these techniques will lead to further structural, mechanical and biophysical investigations of significant amounts of highly purified RUMT and other tRNA modification enzymes.

AN INITIAL ATTEMPT AT DELETING THE *ANX 14* GENE FROM *NEUROSPORA CRASSA*. Carl E. Creutz<sup>1</sup>, Kevin Cartwright<sup>2</sup>, Neil Adesso<sup>2</sup> & Philip Rock<sup>2</sup>, <sup>1</sup>Dept. of Pharmacology, Univ. of Virginia, Charlottesville, VA 22908, <sup>2</sup>Virginia Wesleyan College, Norfolk, VA 23502. We attempted to delete the annexin-encoding *anx 14* gene from *Neurospora crassa*. The annexin gene and flanking genomic DNA sequences were subcloned from a cosmid obtained from the *Neurospora* Genome Project. We generated an annexin knock-out plasmid by replacing the coding region of the annexin gene with a gene for resistance to the antibiotic hygromycin B. Electroporation of the knock-out plasmid into wild-type, hygromycin-sensitive conidiospores generated fifty hygromycin-resistant transformants. PCR analysis of hygromycin-resistant progeny derived from the first twenty transformants indicated that these transformants resulted from ectopic incorporation of the knock-out plasmid into the genome. While most of these ectopic transformants have been phenotypically normal, one class displays a temperature-sensitive hygromycin-resistance. Growth of such transformants is normal in the absence of hygromycin and inhibited by hygromycin below 30 °C. Above 30 °C, exposure to hygromycin is lethal. Such transformants cannot act as the male gamete in a sexual cross but can act as the female gamete. We have yet to obtain a transformant that lacks the annexin gene.

AN EXAMINATION OF MICROORGANISMS AND THEIR ANTIBIOITC RESISTANCE PATTERNS IN RURAL, URBAN, AND TIDAL WATER SAMPLES. Paula B. Lessem, C. Elkins, & K. Hackett, Dept. of Biology, Univ. of Richmond, Richmond, Va. 23173. Water samples from rural (9), urban (3), and tidal (8) sources in eastern Virginia were obtained. Fecal coliforms were isolated using the Coliscan Easygel system and were then screened via minimum inhibitory concentrations (MICs) for resistance to six common antibiotics including erythromycin. Finally, *Escherichia coli* B and *Staphylococcus aureus* ATCC 9144 were grown in membrane-filtered water from the same sites for an approximate period of ten day after which the MICs for the same six antibiotics were repeated. Erythromycin resistance was induced in the *S. aureus* strain following incubation in a filter-sterilized tidal and tap water samples. The MIC for erythromycin in *S. aureus* pre-induction was 0.5 µg/ml. Following induction, the MIC increased to 500 µg/ml (tidal) and 125 µg/ml (tap water). The induced resistance in these water samples indicates that one of four different types of erythromycin resistance genes was turned on by something inherent in the water. RNA from these strains has been isolated and future studies using RT-PCR techniques could potentially determine which genes, if any, were turned on in these strains. Supported by the Undergraduate Research Committee, Univ. of Richmond.

PRELIMINARY CHARACTERIZATION OF *SPY1600*, A PUTATIVE HYALURONIDASE GENE IN *STREPTOCOCCUS PYOGENES*. Karin M. Berling and Wayne Hynes, Dept. of Biol., Old Dominion Univ., Norfolk, VA 23529. *Streptococcus pyogenes* produces many different virulence factors, and the recently completed sequences of two streptococcal genomes (strains SF370 and MGAS8232) have established the existence of several previously unknown genes encoding possible virulence factors. Among these is *Spy1600*, which based on its nucleotide similarity to other hyaluronidase genes, has been proposed to encode a version of this enzyme. The gene has, however, no homology to the hyaluronidase genes in various *Streptococcus* species. The aim of this project is to determine whether *Spy1600* is a hyaluronidase. Primers were designed from a published



sequence, and the gene amplified by PCR. The gene was detected in all 50 strains tested, which indicates a conserved genetic region in the genome. According to the sequence there is no signal peptide within the first forty amino acids of the N-terminal, which would suggest an intracellular product or a non-typical secretion mechanism. The *Spy1600* genes from different strains have been cloned, transformed into *E. coli*, and tested for hyaluronidase activity using a standard hyaluronidase assay. So far, none of the cloned genes have shown activity either intra- or extracellularly. *Spy1600* from strain 10403 has also been sequenced and shows 97% and 98% homology to the genes of SF370 and MGAS8232 respectively.

### Biomedical and General Engineering

**BRAIN REORGANIZATION AND TINNITUS.** Martin Lenhardt, Depts. of Biomedical Engineering and Otolaryngology, Virginia Commonwealth University, Richmond, VA. Adult mammalian brains are not hard wired for life but are very plastic especially in the sensory and motor areas. Recently, brain reorganization has been reported in imaging studies of patients with tinnitus, that is, patients experiencing phantom sound perception. Although tinnitus is often associated with hearing loss it isn't necessary for tinnitus to develop. In fact somatomotor maneuvers can induce or alter tinnitus. A novel treatment approach has been developed based on a central neural site of tinnitus generation due to brain reorganization as a result of auditory and cross modality interactions. The treatment involves to delivery of high frequency patterned stimulation aimed at the reversal of brain reorganization hypothesized as essential for tinnitus generation. Cross modality stimulation was also employed. In a pilot study eight out of nine patients experienced tinnitus suppression immediately after treatment and for most this residual inhibition lasted for days and in a few cases weeks. A demonstration will be provided using a unique piezoelectric actuator that is placed on the skull for bone conduction stimulation.

**INFANT ECHOLOCATOR.** Douglas G. Richards & Martin Lenhardt, Dept. of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA. This project is developing an innovative echolocation device to serve a large population of blind infants who at present have few alternatives to enhance their sensory capabilities and facilitate motor development. The device under development: (a) is the only sensory substitution device for the blind to make use of the human ability to perceive bone conducted high audio (10-20 kHz) and ultrasound (20 - 100 khz); (b) makes use of the natural capacity for echolocation, rather than relying on processed sound, potentially facilitating the learning process, and making a more natural sensory substitution; and (c) by presenting the sounds in the high audio and ultrasonic regions through bone conduction, it does not interfere with hearing of speech or environmental sounds. Feasibility is being demonstrated by a performance evaluation of the sonar parameters relevant to use by a blind infant, e.g. field of view, size and distance of object detection, and of the output to existing data on bone conduction thresholds and frequency discrimination. Alternative modes of coding distance and direction are being explored, including amplitude modulation, frequency modulation, and pulse modulation of high audio and ultrasonic carriers.

**MULTIMODAL INFANT HEARING AID.** Brandon May, Douglas G. Richards, Alan G. Madsen, & Martin L. Lenhardt, Dept. of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA. This project is developing an innovative, vibrotactile/bone conduction aid for hearing impaired infants. The aid is innovative in that it: (a) employs two simultaneous modalities for hearing remediation: vibrotactile and bone conduction, (b) presents both components on the head, an area of the body relevant to speech communication, (c) does not interfere with air conduction hearing, and (d) employs a visual indicator of speech sound level to facilitate linguistic interactions. The project is assessing hardware (e.g., transducers, processors, and amplifiers), developing a speech processing algorithm, and integrating them into a prototype hearing aid. The algorithm separates features of speech (e.g., the fundamental and formants) into appropriate bands for the different transducers. The



long-term objective is to develop a commercial, wearable aid, which will serve a large population of hearing impaired infants who at present have few alternatives for hearing remediation.

PERFORMANCE EVALUATION OF A PACKED-BED BIOREACTOR WITH DOWNSTREAM AMPEROMETRIC DETECTION USING INTERDIGITATED MICROSENSOR ELECTRODE ARRAYS AND GLASSY CARBON ELECTRODES. Kennard M. Brunson, Sean Brahim, & Anthony Guiseppi-Elie, Center for Bioelectronics, Biosensors and Biochips (C3B), Virginia Commonwealth University, Richmond, VA 23284-3038. Flow Injection Analysis (FIA) is a rapidly growing analytical technique that is quickly replacing conventional "beaker chemistry". In this project we evaluated the performance of the amperometric detector of the flow system. Four characteristics of the amperometric response using glassy carbon electrodes were investigated: the peak height, peak area, peak width at  $\frac{1}{2}$  max., and the lagtime between sample injection and signal. The initial analyte chosen was potassium ferrocyanide ranging in concentrations from 0.001M to 0.01M. The data showed a correlation between average peak height and average peak area, with values ranging from 1.0 to 4.5 A and 0.9 to 5.6 A\*min respectively. Lag times ranged between 1.5-2.0 min with an average lag time of 1.7 min while peak widths held steady at ca. 2 min. The second analyte tested was hydrogen peroxide at concentrations from  $2 \times 10^{-7}$ - $10^{-6}$ M. The data also showed a correlation between height and area with values ranging from 1.34-1.54 nA and 5.3-7.6 nA\*min respectively. Lagtime ranged between 1.75-2.05 min an averaged 1.92 min. Widths averaged 1.08 min.

BIO-SMART HYDROGELS: CO-JOINED MOLECULAR RECOGNITION AND SIGNAL TRANSDUCTION IN BIOSENSOR FABRICATION AND DRUG DELIVERY. Sean Brahim & Anthony Guiseppi-Elie, Center for Bioelectronics, Biosensors and Biochips (C3B), Virginia Commonwealth University, Richmond, VA 23284-3038. Two groups of materials that have received widespread attention are hydrogels and conducting electroactive polymers. We have integrated these two materials and physically entrapped enzymes within their matrices to produce novel chemically responsive polymers, which we call electroactive hydrogels. The enhanced biosensing capabilities of these composite films have been demonstrated in the fabrication of glucose, cholesterol and galactose biosensors. All biosensors displayed extended linear response ranges ( $10^{-5}$  –  $10^{-2}$  M), rapid response times (< 60 s), retained storage stabilities of up to 1 year, and exhibited excellent screening of the interferents ascorbic acid, uric acid, acetaminophen and L-cysteine. The cross-linked hydrogel component of these composite films was also prepared with an amine containing methacrylate monomer and entrapped glucose oxidase to produce glucose-response, pH-sensitive polymeric devices. When insulin was subsequently loaded into these "bio-smart" devices, there was a significant increase in insulin release rate when the devices were immersed in glucose solutions.

NEW APPROACH FOR MOLECULAR IMPRINTING. Dmitry Pestov, Natalia Levit, & Gary C. Tepper, Dept. of Chemical Engineering Virginia Commonwealth University, Richmond, VA 23284. We describe a new molecular imprinting technique applied to small particles of monomers with solid-state reactivity. Heptane was used as the print molecule and a Surface Acoustic Wave (SAW) mass sensor was used to detect vapor uptake in the particles. In our imprinting process, the nanoparticles of the monomer (2,5-distyryl-pyrazine or p-phenylenediacrylic acid diethyl ester) were produced by fast expansion of supercritical solution and deposited directly onto the surface of the SAW device. Then, the particles were polymerized at room temperature in the presence of saturated template vapor by UV irradiation. The template molecule was extracted from the particles and molecular imprinting effect was evaluated. Acknowledgements. This material is based upon work supported by the National Science Foundation under grant number 0097409.

SAW BASED CHEMICAL SENSORS: SIGNAL EVALUATION. Bridget Deveney, Gary Tepper, Dmitry Pestov, & Natalia Levit, Dept. of Chemical Engineering, Virginia Commonwealth University, Richmond, VA 23284. Surface Acoustic Wave (SAW) based chemical sensors are extremely sensitive miniature portable chemical sensors. Polymer coatings can be used to make these sensors selective for various chemical vapors. The nature of polymer films makes the signal for these sensors complex and difficult to analyze. Several methods of signal evaluation are compared and their application to differing polymer coatings and solvents are discussed.

INFRARED PARASPINAL TEMPERATURE MEASUREMENT AND HEALTH STATUS. Douglas G. Richards<sup>1,2</sup>, David L. McMillin<sup>2</sup>, Eric A. Mein<sup>2</sup>, & Carl D. Nelson<sup>2</sup>, <sup>1</sup>Dept. of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA and <sup>2</sup>Meridian Institute, Virginia Beach, VA. The purpose of this study was to explore correlations between an objective measurement related to chiropractic and osteopathy - paraspinal temperature variation - and health quality of life. Paraspinal temperature variation has claims for clinical relevance going back to the early days of chiropractic, but there has not previously been any objective confirmation. Temperature imbalances along the spine have been seen as indicators of both somatic and visceral dysfunction, although in the past this has been difficult to quantify. This exploratory study compared paraspinal temperature measurements using the Tytron C-3000 (a computer-interfaced device with paired, infrared sensors) with questionnaire measurements using the SF-36 short form health survey, a well-validated measure of health status. Data from 79 people participating in health assessments were used. The correlations of the SF-36 with measurements of temperature differential on either side of the spine, and temperature variations along the spine, ranged from  $r = -.23$  to  $-.28$ , and were statistically significant at the .05 level. Thus temperature imbalances in the spine are correlated with lower health quality of life.

INFLUENCE OF GEOMETRY OF REPAIRED ARTICULAR CARTILAGE DEFECTS ON CREEP BEHAVIOR DURING INDENTATION. Corrie E. Spoon & Jennifer S. Wayne Orthopaedic Research Laboratory, Depts. of Biomedical Engineering and Orthopaedic Surgery, Virginia Commonwealth University, Richmond, VA 23298. The mechanical properties of articular cartilage are commonly determined using the indentation test. Geometric parameters such as the specimen thickness and indenter radius can vary greatly between indentation applications. This investigation determined whether the geometric parameter variations and degree of integration between the repair and normal cartilage effect the behavior of osteochondral defects and consequently the mechanical properties determined through creep indentation. ABAQUS® finite element analysis simulated the indentation of osteochondral defects with varying ratios of indenter radius to cartilage height ( $a/h=0.5, 1.5$ ) and cartilage radius to indenter radius ( $r/a=2, 5$ ). Fully integrated and non integrated defects were modeled. The vertical displacement under the indenter was curve fit to the biphasic theory to determine the aggregate modulus, permeability, and Poisson's ratio. For specimens with an  $a/h$  of 1.5, changes to the specimen radius ( $r/a$ ) and degree of integration between repair and normal cartilage did not affect the indentation behavior or the mechanical properties determined. Specimens with an  $a/h$  of 0.5, demonstrated a dependence on specimen radius and degree of integration.

NEW TESTING METHOD FOR ASSESSING THE MECHANICAL BEHAVIOR OF ARTICULAR CARTILAGE. Michael J. Araj & Jennifer S. Wayne, Orthopaedic Research Laboratory, Depts. of Biomedical Engineering and Orthopaedic Surgery, Virginia Commonwealth University, Richmond, VA 23298. Mechanical properties of cartilage are required to assess the tissue's ability to function in a joint. Many experimental testing methodologies exist, but few are nondestructive nor suited for in vivo measurements. This study began the development a new nondestructive test to determine cartilage properties that will have application in vivo through arthroscopy. It employed a laser reflectance system to measure cartilage deflection under the action of an aspiration pressure. Experimental measures found deflection of cartilage from a porcine tibial

surface to be ~80microns under an aspiration pressure of 0.1MPa. The experimental configuration was also simulated with a finite element model to predict mechanical behavior. Finite element deflection of the tissue under the same conditions was less than found experimentally. Further improvement of the finite element model will include altering input parameters, boundary conditions, and material symmetry. A combination of experimental testing and theoretical modeling will create a method not currently available to measure cartilage properties in vivo.

**A TECHNIQUE FOR MEASURING FIBULA MOTION RELATIVE TO THE TIBIA DURING TORSIONAL EXPERIMENTS.** John R. Owen<sup>1</sup>, Timothy J. Marqueen<sup>1</sup>, Jennifer S. Wayne<sup>1,2</sup>, & James B. Carr<sup>1</sup>, Orthopaedic Research Laboratory, Depts. of <sup>1</sup>Orthopaedic Surgery and <sup>2</sup>Biomedical Engineering, Virginia Commonwealth University, Richmond, VA 23298. One type of ankle injury results in the tibia and fibula separating (tibiofibular diastasis) causing the ankle to become unstable. Biomechanical evaluations of repair methods should include measurement of tibiofibular diastasis in terms of medial/lateral and posterior/anterior translation, as well as internal/external rotation of the fibula relative to the tibia. Previous studies have not adequately addressed diastasis measurement. Therefore, a device has been developed to measure all three components. A bracket is attached to the fibula and is free to move with it relative to the tibia. A unique arrangement of uni-directional sensors join the fibula bracket to a reference frame that is rigidly attached to the tibia. Instead of directly measuring the three components, the sensors form a combination of triangles that change shape as the fibula moves relative to the tibia. Trigonometric solution of these triangles defines the three components of planar movement, hence diastasis, of the fibula relative to the tibia. This device has since been used to measure tibiofibular diastasis in biomechanical comparisons of repairs using a screw versus a staple.

**EVALUATING THE MECHANICAL AND POLYMERIZATION CHARACTERISTICS OF MODIFIED BONE CEMENTS.** Peter C. Liacouras, Jennifer S. Wayne, & John R. Owen, Orthopaedic Research Laboratory Depts. of Biomedical Engineering and Orthopaedic Surgery, Virginia Commonwealth University, Richmond, VA 23298. Color additives to bone cements for total joint replacements allow for more readily distinguishable cement from surrounding bone tissue. It is unknown whether the additive alters the cement's polymerization characteristics or mechanical properties. This study evaluated several standard acrylic bone cement tests of Endurance, Surgical Simplex P, and a Pigmented Endurance (developed by DePuy, a Johnson and Johnson Company). Dough times for were found to be 3:40, 2:50, and 3:30 (minutes:seconds), respectively. Setting times were 7:35, 10:15, and 9:20. Compression strengths were: 86.30±4.23, 84.17±1.20, 90.67±3.79MPa. Tensile strengths were: 43.68±2.27, 47.30±4.39, and 45.37±2.34MPa. Flexural strengths were: 66.12±4.39, 70.78±6.877, and 66.25±3.69MPa with flexural moduli of 2769.32±85.90, 2728.58±105.07, 2741.03±22.33MPa. Statistical analysis (one-way ANOVA, Tukey's HSD post-hoc comparisons) revealed that the addition of pigment did not affect any of the mechanical properties of Endurance. Analysis revealed statistical equivalence between all cement types, with the exception of Compressive Strength where Pigmented Endurance was stronger than Simplex (p<0.02).

**UTILIZING ELECTROSPINNING TO CONTROL FIBER DIAMETERS OF BIORESORBABLE POLYMERS FOR TISSUE ENGINEERING SCAFFOLDS.** Eugene Boland<sup>1</sup>, Gary E. Wnek<sup>2</sup>, & Gary L. Bowlin<sup>1</sup>, Depts. of <sup>1</sup>Biomedical Engineering and <sup>2</sup>Chemical Engineering, Virginia Commonwealth University, Richmond, VA 23298. Research in the field of Tissue Engineering is striving to develop the ideal scaffold. This ideal scaffold must not hinder the normal function of tissues nor the differentiation and migration of cells yet it must provide adequate support to maintain the proper three-dimensional orientation. To achieve these ends, a scaffold must be penetrable to cells, identifiable as a substitute extracellular matrix (either by size or chemical structure), and provide the mechanical strength to support the developing tissue. These interactions are as critical in determining success or failure of engineered tissues. Utilizing electrospinning of Poly(glycolic acid),

Poly(lactic acid), and Polycaprolactone, we have created fibrous scaffolds that we believe can mimic the needs of the specific target tissues (Patents Pending). We have determined that fiber diameters vary linearly with polymer concentration in the spinning solution (from 100 nanometers to 10 microns), and fiber size, orientation, and spinning solvent control the mechanics of these scaffolds. Various geometries and properties are possible with our prototype electrospinning system in a quick and reproducible manner.

**ELECTROSPINNING OF COLLAGEN NANOFIBERS.** Jamil A. Matthews<sup>1</sup>, Gary E. Wnek<sup>2</sup>, David G. Simpson<sup>3</sup>, & Gary L. Bowlin<sup>1</sup>, Depts. of <sup>1</sup>Biomedical Engineering, <sup>2</sup>Chemical Engineering and <sup>3</sup>Anatomy, Virginia Commonwealth University, Richmond, VA 23298-0694. Electrospinning is a fabrication process that uses an electric field to control the deposition of polymer fibers onto a target substrate. This electrostatic processing strategy can be used to fabricate a wide range of fibrous polymer mats. In this study, we describe how electrospinning can be adapted to produce tissue-engineering scaffolds composed of collagen nanofibers (Patents Pending). Optimizing conditions for calfskin type I collagen produced a matrix composed of 100 nm fibers that exhibited the 67 nm banding pattern which is characteristic of native collagen. The structural properties of electrospun collagen varied with the concentration of the collagen solution used to spin the fibers. Electrospinning is a rapid and efficient process that can be used to selectively deposit polymers in a random fashion or along a predetermined and defined axis. Electrospun collagen promotes cell growth and the penetration of cells into the engineered matrix. In conclusion, the structural, material and biological properties of electrospun collagen suggest that this material may represent a nearly ideal tissue-engineering scaffold.

**EFFECTS OF PARKINSON'S DISEASE AND DEEP BRAIN STIMULATION ON EYE MOVEMENTS AND READING.** Charles Neering, Asma Habib, & Paul A. Wetzel, Dept. of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA 23298. Parkinson's Disease (PD) is a progressive disorder that affects both neuromuscular and oculomotor control. This study was conducted to analyze the effects of deep brain stimulation (DBS) on eye and head movements in PD patients during reading. Both non-PD and PD patients with DBS devices read 10 randomly selected texts of equal character spacing and increasing difficulty level. Eye position was monitored by a pupil-corneal eye tracking system and translated into such parameters as fixation duration and average saccadic amplitude. Head position was sampled by a six-degree of freedom magnetic head tracking system. Periods of fixation instability in PD patients were observed to be associated with uncompensated, uncorrelated movements of the eye and or the head. DBS devices were shown to reduce the frequency of these episodes of instability and improve PD patient reading ability. These episodes can lead to an inability to adequately stabilize fixation resulting in greater reading difficulty and reduced overall quality of life.

**AN EYE MOVEMENT BASED HUMAN MACHINE INTERFACE.** Federico A. Puma & Paul A. Wetzel, Dept. of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA 23298. Certain diseases and disabilities may limit a person's ability to access a computer or to interact with other machines or devices using traditional input devices. A system is described that can be used as a communication aid for people with body or limb movement limitations. An interface is described that incorporates eye and head position measurement and utilizes a combined gaze measurement as an alternative input to control cursor movement on a computer. Transfer from mouse-cursor control to gaze-cursor control is done using a keyboard key. The system has been designed to operate in the background with other Windows based applications and will be evaluated for clinical and rehabilitation use.

PERFORMANCE OF ARTIFICIAL NEURAL NETWORKS IN THE PREDICTION OF LOWER TORSO MUSCLE RECRUITMENT PATTERNS. Miguel A. Perez & Maury A. Nussbaum, Grado Dept. of Industrial & Systems Eng., Va. Polytechnic Inst. & State Univ., Blacksburg, VA 24061. Prediction of muscle recruitment patterns in the lower torso is useful in the modeling of low-back injury risk, especially in occupational manual materials handling tasks. While several mathematical models are available to predict muscle recruitment patterns, some disadvantages specific to the use of each model exist. Artificial Neural Networks circumvent many of these disadvantages and have been successfully used in problems of a similar nature. However, verification of the network's predictive performance had been somewhat limited in its scope. This investigation expanded the verification of a network's performance to high-magnitude static torso exertions involving tri-axial moments. Low levels of predictive performance (average  $R^2$ s in the 0.20s) were initially observed, and prompted further experiments to understand the factors affecting the network performance. The results of these experiments indicate that the main sources of prediction error are the physiological parameters (e.g. muscle moment arms, maximum muscle stress) that have been used in the model. Current research efforts are being directed to identify and correct those parameters that generate substantial amounts of error in model predictions.

NEURAL NETWORK DESIGN OF A DISHWASHER CONTROL BOARD. Michael Morris & Rosalyn S. Hobson, Dept. of Electrical Engineering, Virginia Commonwealth University, Richmond, VA 23220. In this paper a novel approach to designing an advanced control board for use in appliance, and specifically dishwasher, control. A software model of a typical control board is constructed using Simulink to understand the control path to be designed, and is used as a benchmark for an innovative controller. Hardware control of the dishwasher is realized by use of the Invensys Control Systems tool used for hardware design. The 'brain' of the dishwasher is a neural network using the Widrow/Hoff learning method. Smart control is achieved using sensors, timing mechanisms, and the Widrow/Hoff neural network.

CLASSIFICATION OF VISUAL SEARCH USING NEURAL NETWORKS. Harsh Raju & Paul A. Wetzel, Dept. of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA 23298. Visual search describes the process of how the head and eyes move in a visual field in order to acquire a visual object. A neural network based approach has been implemented to assess and objectively compare visual search patterns within and between observers. A hybrid Learning vector quantization neural network has been developed to perform translation, rotation and scale invariant (TRSI) visual search classification. Unrestricted head movement data collected during visual search of a stationary target in a hanger under different viewing conditions namely simulated daytime with unrestricted field of view (FOV), simulated daytime with 40° FOV and using night vision devices were analyzed. The neural network is coupled to a preprocessing stage that extracts features of the search pattern that are TRSI. A supervised learning rule, Learning vector quantization1 (Lvq1) was used to train the network. The network was able to classify the patterns with 90% accuracy under TRS conditions. Patterns were quantified and compared through computation of the correlation coefficient. A highly accurate neural network, vision model was developed and visual search was successfully quantified.

AN ANALYSIS OF FEEDING BEHAVIOR IN PRE-MATURE INFANTS. Patrick M. Boland<sup>1</sup>, Paul A. Wetzel<sup>1</sup>, Rita H. Pickler<sup>2</sup>, & Rosalyn S. Hobson<sup>3</sup>, <sup>1</sup>Dept. of Biomedical Engineering, <sup>2</sup>School of Nursing, and <sup>3</sup>Dept. of Electrical Engineering, Virginia Commonwealth University, Richmond, VA 23298. Premature infants, by definition, are neurologically underdeveloped. They have difficulty or inability in bottle-feeding which is manifested by a disorganized "suck, swallow and breathe" complex. The Pre-Term Readiness & Outcomes Study (PRO Study) tests a predictive model of bottle-feeding readiness and bottle-feeding outcomes and uses biomedical signal analysis as one of the research components. The analog signals collected include sucking (strain gauge), swallowing (EMG), respiration (piezoelectric nasal sensor), ECG, and oxygen saturation and pulse wave via pulse

oximeter. A feed-forward, multi-layer perceptron configuration using a backpropagation algorithm was implemented to differentiate between two of the data signals collected - suck and swallow - with only limited success. A Learning Vector Quantization (LVQ1) neural network algorithm was then used to analyze a series of two-second suck and swallow samples (targets) obtained at a 500-Hz sample rate. Eighty or fewer epochs with a learning rate of 0.01 were required to consistently train a network able to identify the test data with an accuracy of 100%.

**SPEECH PROCESSING IN HIGH NOISE.** Douglas G. Richards, Martin L. Lenhardt, & Alan G. Madsen, Dept. of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA. Speech intelligibility is a problem in high noise environments (e.g., aircraft carrier flight decks). Although active noise cancellation technology can reduce the noise at low frequencies, it is of little use in the higher speech frequencies, where low-energy consonants convey much of the meaning in speech. We have developed digital signal processing algorithms for moving the consonants into a higher frequency region that is less susceptible to noise, and presenting those frequencies through bone-conduction transducers directly to the inner ear. The processing includes filtration to isolate the consonants, and modulation of carriers in the high audio (10-20 kHz) or ultrasonic (20-100 kHz) range. Bone conduction is accomplished through custom piezoelectric transducers and amplifiers.

### Botany

**THE FLORA OF VIRGINIA PROJECT: AN UPDATE.** Marion B. Lobstein, Dept. of Biology, Northern Virginia C.C., Manassas, VA 22205. Virginia, for its landmass, has the most diversity of species of vascular plants of any state. It had the first flora, the *Flora Virginica* in 1739 by John Clayton, yet does not have a modern flora of Virginia. The Virginia Academy of Science for over fifty years has supported efforts to produce a modern flora. In 1999, renewed efforts to produce a modern flora were made through the VAS Botany Section and the Virginia Flora Committee. Financial support from VAS followed. In 2001 the Foundation of the Flora of Virginia, Inc. was formed to be the primary vehicle for the development and production of the *Flora of Virginia*, and its Board of Directors met in August, 2001. In early 2002 a Flora Advisory Board was formed, made up of 50 botanists from around the Commonwealth (including many VAS members). It met in February to develop guidelines for the format and other details of the *Flora of Virginia*. Alan Weakley, Curator of the UNC-Chapel Hill Herbarium, will be the first author of the *Flora*. Chris Ludwig, Division of Natural Heritage, is Executive Director of the Project and will be the second author. He will be permitted to direct the Project as part of his position duties. Federal tax-exempt [501(c)3] status has been granted for the Project and serious fund-raising and public outreach are going forward.

**ACALYPHA DEAMII EAST OF THE APPALACHIANS.** Patricia A. Truman<sup>1</sup>, Gary P. Fleming<sup>2</sup>, & W. John Hayden<sup>1</sup>, <sup>1</sup>Department of Biology, University of Richmond, VA 23173 and <sup>2</sup>Virginia Department of Conservation & Recreation, Division of Natural Heritage, 1500 E Main St., Richmond, VA 23219. Until recently, *Acalypha deamii* was thought to be a relatively rare species restricted to flood plains of the Ohio and mid-Mississippi River systems. We report the presence of this frequently overlooked species in Virginia, Maryland, and eastern West Virginia. Since 1997, our field work has amassed over 50 collections of *A. deamii* from floodplain forests of the James, Roanoke/Stanton, Rappahannock, Shenandoah, and Potomac rivers. Typical habitat for *A. deamii* has considerable microtopographic diversity including periodically flood-scoured areas with high surface cover of exposed sandy soil. These soils are moderately acidic but fertile with moderately high calcium, magnesium, and manganese levels and high total base cation saturation. Frequent associates are annual herbs and delicate perennials. Along with *A. virginica*, *A. rhomboidea*, and *A. gracilens*, *A. deamii* is a member of the so-called *Acalypha virginica* complex resembling most closely the

widespread and weedy *A. rhomboidea*. *Acalypha deamii* can be recognized by its robust stature, broad leaves, 2-carpellate gynoeceia, large (2.2-3.2 mm) seeds, and allomorphic flowers and fruits.

INITIATION AND FIRST RESULTS OF A FLORISTIC INVENTORY FOR RANCHO KIUIC, YUCATAN. W. John Hayden, Department of Biology, University of Richmond, VA, 23173. The opportunity to document vascular plant diversity at the Helen Moyers Bio-Cultural Reserve located at Rancho Kiuic, Yucatan, Mexico, grew from the author's participation in an environmental citizenship experiential learning course taught in collaboration with colleagues at Millsaps College and the Autonomous University of Yucatan (UADY). Reported here are initial stages in the development of the inventory. Rancho Kiuic is located within the Puuc Hills, a region of modest relief within the otherwise very flat Yucatan peninsula. Throughout the region, much of the forest is maintained at early successional stages because of clearing for pasture or swidden agriculture. The forest at Kiuic, however, is older and more mature than that of most of the surrounding area. So far, field work has sampled vegetation in December (dry season), early June (cusp between dry and wet seasons), and late July (wet season). Presently the inventory consists of 214 species documented with vouchers at UADY and/or UR. Leguminosae and Euphorbiaceae are particularly abundant. The flora is being documented via digital photography and early stages of development of photographic aids to plant identification are being developed.

AN INTERNET-BASED INVENTORY OF THE TREES AND SHRUBS OF THE UNIVERSITY OF RICHMOND CAMPUS. Tihomir S. Kostadinov & W. John Hayden, Department of Biology, University of Richmond, VA 23173. An Internet-based resource for the woody dicotyledonous plants growing on the University of Richmond campus was created during the summer of 2001. Both exotic and native trees and shrubs were included in the study. More than 170 woody dicotyledonous species were documented, in 44 angiosperm families. Each species is represented by a unique page including one or more high-resolution digital photographs accompanied by a brief botanical description derived from standard literature. Herbarium specimens were collected for each species; these vouchers are identified on the website and are located at the University of Richmond Herbarium (URV). In addition, the web site features a brief introduction to the physical environment of the UR campus, some statistics, and lists of species sorted by family, genus, and common name. The web site has many potential uses for pedagogy in biology, botany, horticulture, and natural history. It is available to the public at: <http://www.mathcs.richmond.edu/~tkostadi/trees/>

A COASTAL FOREST'S RESPONSE TO RISING SEA LEVEL ON THE DELMARVA PENINSULA, MARYLAND. M. L. Kirwan, College of William and Mary, Williamsburg, VA 23186 and J. L. Kirwan & C. A. Copenheaver, College of Natural Resources, Virginia Tech, Blacksburg, VA 24061. To address the impact of rising sea level on a Maryland coastal forest, 15 loblolly pines (*Pinus taeda*) were cored for dendroecological analysis. The studied forest is a pure stand of loblolly pine that extends down an elevation gradient into surrounding marsh. Dead stumps and snags in the marsh indicate a retreating forest margin. Although relative sea level has risen considerably, there is no associated decline in ring width and likely no associated mortality. Instead, ring width is correlated positively with annual precipitation and winter temperature and negatively with summer temperatures. Although recruitment of new individuals was continuous between 1910 and 1930, there has been no more active recruitment except for a small age class established immediately after regional drought. Because recruitment is failing in the present forest despite abundant seedlings and a loose canopy, recruitment ability appears to be limited by saturated soils associated with periods of high sea level. The forest margin will retreat stepwise, following storm induced mortality or continuously, following natural adult mortality. The position of the forest margin is then a function of sea level position, but it represents the failure to recruit new individuals, not the ability of adults to survive.

COMPARISON OF NUCLEAR- AND CHLOROPLAST-BASED PHYLOGENIES IN *ILIAMNA* (MALVACEAE). Tracey A. B. Slotta and Duncan M. Porter, Dept. of Biology, Virginia Polytechnic Inst. & State Univ., Blacksburg, VA. Sequences from the internal transcribed spacer (ITS) of the nuclear ribosomal region and the trn L-F spacers of the chloroplast genome were used to resolve relationships in *Iliamna* Greene (Malvaceae). The eight species of *Iliamna* were included as well as representatives from *Malacothamnus*, and *Phymosia*. *Iliamna*, *Malacothamnus*, and *Phymosia* are thought to comprise the *Malacothamnus* Alliance based on chromosome numbers and morphological similarities. Preliminary results in the present study indicate a strong affinity between *Iliamna*, *Malacothamnus*, and *Phymosia*. Sequences from the ITS region of *Iliamna* contained several insertion/deletion events preventing direct sequencing; whereas, in *Malacothamnus* and *Phymosia*, these events were not detected. This finding supports the hypothesis that *Iliamna*, with a base chromosome number of  $n = 33$ , is a polyploid possibly derived from either *Malacothamnus* or *Phymosia*, in which  $n = 17$ . Topology of the phylogenies was examined and a few rearrangements of clades were found. It appears that *Iliamna* may not be monophyletic, and that a few species may belong in another genus. Additional sampling from a wider variety of potential relatives is required before a clear picture of the evolutionary history of the genus can be developed.

NEUSTON PHYTOPLANKTON: A UNIQUE COMMUNITY WITHIN AQUATIC HABITATS. Harold Marshall<sup>1</sup> and Lubomira Burchardt<sup>2</sup>, Dept. Biological Science, <sup>1</sup>Old Dominion University, Norfolk, VA, Dept. Hydrobiology & <sup>2</sup>Adam Mickiewicz Univ., Poznan, Poland. The algal populations within the neuston surface micro-layer (2-3 mm in depth) using a glass plate collection protocol, were collected from a small (0.23 ha) pond and Lake Drummond in southeast Virginia. Collections were made between August 2001 and March 2002, with sub-surface phytoplankton collected for comparison. The pond contained a diverse representation of species (39) in the neuston, with the flora dominated by blooms of chlorophytes (*Scenedesmus* spp.) in August, diatoms (*Nitzschia acicularis*) in January, and cyanobacteria (*Aphanizomenon flos-aquae*) in March. There was less diversity (23 species) in Lake Drummond neuston which was dominated by a variety of diatoms, which produced a bloom of *Asterionella formosa* during January and February, with other diatoms also dominant (e.g. *Aulacoseira herzogii*). Total neuston concentrations ranged from 0.5 to  $1.3 \times 10^3$  cells l<sup>-1</sup> in the Lake Drummond, with sub-surface phytoplankton concentrations at 0.2 to  $2.7 \times 10^3$  cells l<sup>-1</sup>. Total neuston in the pond ranged from 16.5 to  $57.0 \times 10^3$  cells l<sup>-1</sup>, with sub-surface neuston concentrations at 19.4 to  $40.4 \times 10^3$  cells l<sup>-1</sup>. In general, similar taxa were observed in both the neuston and sub-surface collections.

HISTORICAL ENVIRONMENTAL CHANGE IN LAKE DRUMMOND OF THE GREAT DISMAL SWAMP. Jeremy L. Hicks & Jennifer E. Slate, Dept. of Biol., Old Dominion Univ., Norfolk, Va 23529. Lake Drummond of the Great Dismal Swamp is one of two naturally occurring lakes in Virginia. The water is highly acidic and tea-colored due to the great influx of organic matter. To determine water quality change in Lake Drummond since European settlement, a variety of paleoecological techniques will be employed. From preliminary microfossil diatom analysis and organic/inorganic composition of a 19-cm sediment core, it is hypothesized that deforestation began at 12-13 cm depth. Benthic diatom species increased and inorganic content (possibly sand) was high. At 4-5 cm depth, another peak in inorganic mass occurred, which was possibly caused by increased erosion due to canal digging. This increase in erosion could have caused nutrient concentrations to increase in the lake. More data will be obtained, including <sup>14</sup>C and <sup>210</sup>Pb dating of sediment, inorganic and organic sediment chemistry, and sediment grain-size analysis. Analysis of chrysophyte cysts, sponge gemmoscleres, and plant phytoliths may also prove valuable.

SPECIATION IN AQUATIC PLANTS: A CASE STUDY IN WATER LILIES. Kristi Niehaus, Khidir W. Hilu, Dept. of Biol., Virginia Polytechnic Inst. & State Univ., Blacksburg, VA 24060, & Thomas B. Borsch, Dept. of Systematics and Biodiversity, Univ. of Bonn, Germany. *Nymphaea odorata* is the most common water lily in North America. A recent classification accepts two



subspecies: subsp. *odorata* and subsp. *tuberosa*. The closest relative of *Nymphaea odorata* is *N. mexicana*, which overlaps with subsp. *odorata* from Texas to Florida. Subspecies *tuberosa* occurs further north. We studied patterns of variation and speciation in *N. odorata* using morphology and sequence data from ITS and *trnT-F*. We sampled 43 populations across the range of *N. odorata* and four *N. mexicana* populations. We examined 40 morphological characters with univariate analysis, SAHN clustering, and PCA. Morphology was highly variable, with no significant grouping by either subspecies or geography. ITS sequences revealed the potential presence of different paralogues and point to gene flow between and within the two subspecies. Subspecies *tuberosa* seems to be characterized by a different cpDNA haplotype. Parsimony and NJ analyses of nrDNA sequence data excluding the apparently heterozygous individuals revealed two clades representing the two subspecies.

PRELIMINARY ANALYSIS OF ISSR BANDING PATTERNS IN THE *SILPHIUM ASTERISCUS* COMPLEX. Jennifer A. Clevinger, Curtis C. Clevinger, Steven M. Bernacki, Jennie H. Fairservice & Cassidy L. Turner, Department of Biology, James Madison University, Harrisonburg, VA 22807. The genus *Silphium* L. is a member of the sunflower family Asteraceae. It is primarily distributed in the eastern United States. Previous phylogenetic analyses support the division of *Silphium* into two sections, *Silphium* and *Composita*, based upon DNA sequences and growth form. Within these two sections, some clades lacked sufficient variation in the internal transcribed spacer (ITS) and external transcribed spacer (ETS) regions of nrDNA for resolution. One such clade contained two Appalachian endemics, *S. brachiatum* and *S. mohrii*, and the geographically widespread *S. asteriscus* complex. Four additional cpDNA regions have been preliminarily sequenced for this clade but were found to have less than 0.1% variation. Currently, samples from the *S. asteriscus* complex, *S. mohrii* and *S. brachiatum* are being assayed for inter-simple sequence repeat (ISSR) regions. Six of ten primers tested so far have shown variation and are being analyzed. The ISSR banding patterns will allow us to resolve relationships within this poorly understood clade.

IN VITRO REGENERATION OF *SAINTPAULIA RUPICOLA*. Michael H. Renfroe & Evonne N. Johnson, Dept. Biol., James Madison Univ., Harrisonburg, VA 22807. Kenya is home to the endangered species of African violet *Saintpaulia rupicola*. Loss of habitat and changing environmental conditions threaten to eliminate this species from the wild. One approach to conserving this species and the genetic resources that it possesses is to clonally propagate members of this species without removing the original plants from the wild. In order to accomplish this task, conditions required for in vitro regeneration of this species must be understood. To determine the requirements for in vitro clonal propagation, we collected axenic leaf explants and placed them on a tissue culture medium containing various combinations of benzylaminopurine (BAP) and naphthaleneacetic acid (NAA). Best shoot regeneration occurred when BAP was in high concentration relative to NAA. Media discoloration occurred during the culture period. Attempts were made to reduce media discoloration by inclusion of ascorbic acid, polyvinylpyrrolidone, or activated charcoal. Only activated charcoal reduced discoloration, but shoot regeneration also declined with this treatment. Differential responses were noted among genotypes tested. Results indicated that genetic variation was present among the sample population and that in vitro propagation was feasible for this species of African violet.

ANALYSIS OF INTERSPECIFIC AND INTRASPECIFIC INTERACTIONS BETWEEN *AILANTHUS ALTISSIMA* AND *ROBINIA PSEUDOACACIA*. Lara J. Call & Erik T. Nilsen, Dept. of Biol, Virginia Tech, Blacksburg, VA 24060. The exotic invasive *Ailanthus altissima* and the native *Robinia pseudoacacia* are frequently found in disturbed sites, exhibit similar growth and reproductive characteristics, yet have distinct functional roles such as allelopathy and nitrogen fixation, respectively. 1) A full additive series in the greenhouse and 2) spatial point pattern analysis of trees in the field were used to analyze the intraspecific and interspecific interference between these species. In the greenhouse, total biomass responses per plant for both species were significantly affected by

interspecific interference. Indices such as Relative Yield Total and Relative Crowding Coefficient suggested that *A. altissima* was the better competitor in mixed plantings for above ground and below ground interactions. Yet, *R. pseudoacacia* had larger aboveground relative yield in high density mixed pots and greater mean biomass responses for multiple biomass traits. Analysis of spatial point patterns in the field indicated that the two species were positively associated along highly disturbed skid trails in the majority of the field sites. Locally, increased disturbances could lead to more opportunities for *A. altissima* to invade, negatively interact with *R. pseudoacacia*, and become established in place of native species.

THE USE OF REAL-TIME MOLECULAR DIAGNOSTICS FOR THE RAPID DETECTION OF THE DINOFLAGELLATES *PFIESTERIA PISCICIDA* AND *P. SHUMWAYAE*. Sheryl Lynne Walton, Wayne Hynes, & Harold Marshall. Dept. of Biol. Sci., Old Dominion Univ., Norfolk, Virginia 23529. *Pfiesteria* spp. are toxic dinoflagellates that have been found to be associated with fish-kills in rivers and estuaries in North Carolina and other areas along the east coast of the United States. The dinoflagellates have also been found to be associated with human illness, specifically Possible Estuary-Associated Syndrome (PEAS). Due to the public health ramifications that the presence of toxic dinoflagellates may have on a fishing or recreational community, the CDC implemented a monitoring of the various rivers and estuaries along the southeast coast of the Atlantic Ocean for *Pfiesteria* and *Pfiesteria*-like organisms beginning in June 1998. In the past, more traditional methods for detection of the various dinoflagellates were labor-intensive and timely to ascertain the causative agent for a fish-kill; however, in order to increase the public health response to the presence of toxic dinoflagellates, more rapid forms of detection were developed by various laboratories. For a more rapid detection of *Pfiesteria* spp. and *Pfiesteria*-like organisms in the various rivers and estuaries along the Chesapeake Bay, our lab has adapted and implemented molecular techniques, including quantitative and multiplex real-time quantitative assays. Here, we present the development and sensitivity for each of these assays for the various dinoflagellates. This work was supported by VDH and CDC.

DISTRIBUTION OF TREE SPECIES ALONG A FLOODING GRADIENT IN ARKANSAS COASTAL PLAIN SWAMPS. Edward E. Dale, Jr., Dept. of Biol. Sci., Univ. of Arkansas, & Stewart Ware, Dept. of Biol., Col. of Wm. & Mary. In floodplains of the Gulf Coastal Plain, Arkansas Valley, and Mississippi Valley in Arkansas, transects were run from sloughside or streamside outward until upland forest was encountered. First rise to dominance was recorded for each dominant tree species along each transect. By comparison of transects, elevational order of rise to dominance (= presumed flooding tolerance) could be ascertained for 20 species, with classification as backwater, riparian, or both. The backwater sequence was baldcypress (wettest), water tupelo, buttonbush, water elm (*Planera*), swamp privet (*Forestiera*), water locust, overcup oak, water hickory, Nuttall oak, green ash, southern hackberry, American elm, boxelder, sweetgum, willow oak, water oak, cherrybark oak, and shagbark hickory. The riparian sequence was black willow, buttonbush, cottonwood, water hickory, green ash and thereafter like the backwater sequence. In 58 quantitatively sampled stands, shagbark hickory, boxelder, and southern hackberry were important in wetter sites than predicted by transect data. Cedar elm far exceeded American elm in abundance. Red maple, swamp chestnut oak, black gum, laurel oak, and pumpkin and Carolina ashes were absent or rare at all sites.

SCANNING ELECTRON MICROSCOPIC EXAMINATION OF A PFIESTERIA-LIKE DINOFLAGELLATE. Mikolaj Kokocinski and Harold Marshall. Dept. Biological Science, Old Dominion University, Norfolk, VA 23529-0266. The thecal plate tabulations for two dinoflagellates identified as belonging to the *Pfiesteria*-like complex were examined under SEM and compared to *Pfiesteria piscicida* and *P. shumwayae*. Cell cultures CCMP-1833 and CCMP-1838 were obtained from the Center for Culture of Marine Phytoplankton (CCMP). *Pfiesteria* spp. cultures was provided by Dr. JoAnn Burkholder (NCSSU). Sub-cultures were established in our laboratory and fed

*Rhodomonas* sp. (CCMP-757) in filtered and autoclaved sea water (10-15 ppt) in an incubator at 24.5°C and a 12:12 hour light:dark regime. A suture-swelling technique (Truby, 1997) was used for SEM examination. The plate patterns for CCMP-1833 and CCMP-1838 were similar, being: 4', 2a, 6'', 6c, 4s, 5''', 0p''', 2'''. However, there was a distinct difference in the size of the diamond-shaped intercalary plates, with larger anterior intercalaries (1a, 2a) present in CCMP-1838. In CCMP-1838, these anterior intercalaries (1a, 2a) are much larger than in CCMP-1833. Also, the two taxa differ in size, with CCMP-1833 < 15 µ and CCMP-1838 > than 15 µ. Neither CCMP-1833 or CCMP-1838 had plate patterns and tabulations similar to *Pfiesteria* spp.

RESULTS OF THE VIRGINIA MONITORING PROGRAM FOR PFIESTERIA SPECIES AND PFIESTERIA-LIKE ORGANISMS (PLO). M. Braynard, J. Hicks, T. Stem, T. Egerton, J. McNally, C. Taylor, S. Walton, M. Kokocinski, & H.G. Marshall. Dept. Biol. Sci., Old Dominion University, Norfolk, VA 23529-0266. The *Pfiesteria* spp. and *Pfiesteria*-like organisms have been monitored monthly and/or twice monthly from over 100 Virginia estuarine sites from May through October since 1998. Cells from water samples with high PLO counts are established in clonal cultures, examined with scanning electron microscopy for plate tabulation, and used in fish bioassays to determine toxicity. Final confirmation is conducted through PCR analysis, with cross-confirmation of species identified with laboratories at NCSU, UNC, and UMD. PLO occurred in over 90% of the samples and concentrations ranged from 0 to 49,000 cells/ml. The most common PLO were *Gyrodinium* spp., *Cryptoperidiniopsis* spp., *Gymnodinium* spp., and *Karlodinium micrum*. Highest cell concentrations occur from May through July, in waters of 10-20 ppt, and temperatures >20°C. In 2001, over 1200 water samples were analyzed during this period. From these, *P. shumwayae* was identified in the Pagan River and East River, with *P. piscicida* not present in the samples. However, toxic strains of both *Pfiesteria* spp. and various PLO have been maintained in our laboratory for life cycle and bioassay studies.

STATUS AND LONG-TERM TRENDS IN PHYTOPLANKTON POPULATIONS IN THE CHESAPEAKE BAY AND SEVERAL VIRGINIA TRIBUTARIES. T. Stem, J. McNally, J. Hicks, M. Braynard, T. Egerton, K. Alperin, M. Lane, K. Nesius, and H.G. Marshall. Dept. of Biological Science, Old Dominion University, Norfolk VA, 23529-0266. Based on a 15 year data set, these waters continue to be dominated by a diatom flora, which have major growth pulses in spring, summer, and fall. The summer flora is composed of an assemblage of diatoms, cyanobacteria, and phytoflagellates. Potential toxin producing species (14) have been identified and are being monitored. There are increased trends in total phytoplankton abundance and biomass, with mixed patterns among the various nutrients. There is a decreasing trend in productivity over this period (1985-2001), which is accompanied at various sites by increased TSS and reduced Secchi readings. No significant trends were associated with various phytoflagellates, cyanobacteria, or the autotrophic picoplankton. However, of concern in both the Bay and tributaries in recent years is an increased abundance of cyanobacteria and the frequent occurrence of dinoflagellate blooms within the lower reaches of the rivers. Most recently (2002) there was the extended bloom of *Dinophysis acuminata* (a toxic dinoflagellate) in several Virginia inlets of the Potomac River.

## Chemistry

SYNTHESIS AND CHARACTERIZATION OF COMPLEXES OF Co(II), Ni(II), Cu(II) AND Zn(II) WITH AMIC ACID LIGANDS AS MOLECULAR MODELS FOR METAL-DOPED POLYIMIDES. Takyia Ahmed, D.L. Polo, L.M. Vallarino & J.W. Williams, Department of Chemistry, Virginia Commonwealth University, Richmond, VA 23284-2006. This work is part of an ongoing class project that investigates the coordinating ability of the amic acid sites of polyimides through a study of the metal complexes of representative monomeric amic acid models. The ligands, N-(2-methylphenyl)phthalamic acid (H-oMeNPPA) and N-(2-methoxyphenyl) phthalamic acid (H-

oMeOxNPPA), were synthesized by condensation of phthalic anhydride with the appropriate substituted aniline. They were then reacted with the metal acetates to yield complexes of the general formula  $ML_2 \times (\text{solvent})_n$ , where  $n = 0.5 - 1.5$  of the solvent acetic acid, water and/or ethanol. The complexes of Co(II), Ni(II) and Zn(II) had identical IR spectra and solubility patterns. On the basis of the d-d electronic spectra of the Co(II) and Ni(II) species, and of the  $^1\text{H}$  NMR spectrum of the Zn(II) species, these complexes were assigned a six-oxygen-donor octahedral geometry with the amic acid anions acting as ligands *via* the carboxylate groups. The less soluble Cu(II) complex, which had a somewhat different IR spectrum, was instead assigned a dimeric structure with bridging carboxylates.

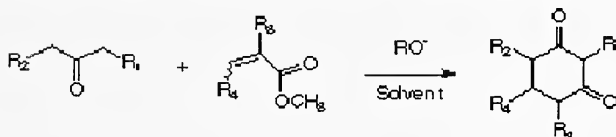
TEMPERATURE PROGRAMMED DESORPTION OF PRIMARY ALCOHOLS FROM ALUMINA. Thomas C. DeVore, Dept. of Chem., James Madison University, Harrisonburg VA 22807. The desorption of methanol, ethanol, 1-propanol, 1-butanol, and 1-pentanol from calcined boehmite in vacuum at temperatures between 300K and 700 K was investigated using temperature programmed desorption - FTIR spectroscopy. The desorption pattern was similar for all of the alcohols tested. The alcohol began desorbing at temperatures as low as 300 K, the ether begins desorbing at  $\sim 450$  K, and the 1-alkene is first observed at  $\sim 525$ K for all alcohols except methanol. The kinetics for each process has been determined. Semi-empirical calculations ( $\text{PM}_3$  level) for methanol on a model boehmite surface indicated that the alcohol binds to the aluminum atoms on the surface. Dissociation of the alcohol on adjacent acid-base sites is energetically favorable and could be a step in the mechanism leading to the observed products. This project was funded by NSF-CHE-0076685, NSF-REU-0097448, and NSF-REU-0097449.

SYNTHESIS OF 3-ACYLINDOLES. Saeid Esmailian & Wayne M. Stalick, Department of Chemistry, George Mason University, Fairfax, VA 22030-4444. The development of a two-step approach to the synthesis of direct  $\gamma$ -carboline precursors is a topic of interest in our laboratory. It is possible to make 3-acylindoles in one step from indole and acyl halides using a mild Friedel-Crafts catalyst, in this case,  $\text{SnCl}_4$ . A series of these 3-acylindoles have been synthesized so they possess a variety of pendent groups, that will eventually end up as 1-position substituents in the final  $\gamma$ -carboline products. 3-acylindoles exhibit biological activities. Many simple indole derivatives show predominately excitatory actions, and a considerable number of 3-acylindoles reportedly show anticonvulsant actions. In the synthesis, whenever the Lewis acid is added to the reaction mixture containing indole and the acylating agent, a strong color change occurred. Under such Friedel-Crafts condition indole trimers and monoacylated indole trimers are produced along with a minor quantity of 3-acylated indole. Changing the addition order, adding  $\text{SnCl}_4$  to a solution of only indole in  $\text{CH}_2\text{Cl}_2$  at  $0^\circ\text{C}$  followed by the addition of acylating agent, allowed for the production of 3-acylindoles as the major product. Another change, addition of nitromethane as a co-solvent, helps to reduce the reaction time. It also increases the solubility of the solid indole-Lewis acid complex, resulting in increased yield.

PROGRESS IN DEVELOPMENT OF NOVEL OXIDATIVE PROCESSES. Christopher T. Lloyd, James H. Wynne & Robert F. Cozzens, Chemistry Division, Naval Research Laboratory, 4555 Overlook Ave, Washington, DC 20375. In the past few years, we have sought to further develop an environmentally friendly, atom-economical methodology for the oxidation of primary and secondary alcohols to their corresponding aldehydes and ketones. Such a method would be ideally suited for many industrial processes. We wish to discuss our recent findings *en route* to optimizing this oxidative methodology. The optimization process involves investigating the effects of varying catalyst/co-catalyst and oxidant concentrations, as well as reaction times and temperatures. Several organometallic transition metal catalysts are employed along with a variety of phase transfer catalysts. Hydrogen peroxide and air are employed as the oxidants in the process. The stability and longevity of the metal catalyst is proportional to the concentrations of the PTC; lesser amounts of PTC result

in catalytic deactivation and poisoning. Recent advances and scale-up potential are discussed in detail.

**REGIOSELECTIVE ROUTES TO VARIOUSLY SUBSTITUTED CYCLOHEXANE-1,3-DIONES.** Samuel A. Simpson, Belhu Berhanu & Godson C. Nwokogu, Dept. of Chem., Hampton Univ., Hampton, VA 23668. Cyclohexane-1,3-diones are versatile starting materials for the syntheses of complex aromatic and non-aromatic organic molecules. Up to four substituents can be introduced on the ring during its assembly from appropriately substituted ketones and acrylate ester. This one-pot synthesis led to inseparable mixtures of regioisomers with unsymmetrical ketones but was a clean, high yield reaction with symmetrical ketones. For pure samples of unsymmetrically substituted cyclohexane-1,3-diones, a multi-step route involving a synthetic equivalent of unsymmetrical ketones, was shown to lead to a single regioisomer, with many of the individual steps being high yield reaction.



**EVALUATION OF STATE OF THE ART ANALYTICAL TECHNOLOGIES FOR THE STUDY OF PESTICIDES AND OTHER PERSISTENT ORGANIC POLLUTANTS.** Gervais E. Assey & Isai T. Urasa, Dept. of Chemistry, Hampton University, Hampton, VA 23668. The research presented in this paper is a comparative evaluation of the analytical capabilities of modern analytical technologies for measurements of persistent organic pollutants (POPs), with emphasis on selected pesticides. The analytical techniques of interest include: High Performance Liquid Chromatography coupled with Diode Array Detector (HPLC-DAD), High Performance Liquid Chromatography coupled with Mass Spectrometric Detector (HPLC-MSD), Gas Chromatography coupled with Flame Ionization Detector (GC-FID), and Gas Chromatography coupled with Electron Capture Detector (GC-ECD). The analytes of interest represent three classes of pesticides, namely: organophosphates, organochlorides, and carbamates. The aim is to determine the optimal measurement parameter requirements, detection capabilities, and measurement selectivity. For purposes of enhancing measurement sensitivity, the research has also integrated solid phase extraction (SPE) with the analytical technologies in question, utilizing reversed phase stationary phase and locally developed biosolids. The results obtained to date indicate that the three classes of pesticides differ significantly with respect to their response to each of the analytical technologies. Furthermore, while organophosphates and carbamates appear to be quite responsive to preconcentration, achieving two-to-three orders of magnitude improvement in detection limits, the organochlorides show very little response to preconcentration.

**SYNTHESIS OF THIOLS FOR CHEMICAL SENSOR APPLICATIONS.** James H. Wynne, Christopher T. Lloyd & Arthur W. Snow, Chemistry Division, Naval Research Laboratory, 4555 Overlook Ave, Washington, DC 20375. In light of recent events, the demand for novel chemical micro-sensors has expanded exponentially. We report the synthesis of a unique series of functionalized thiols for use in chemical sensor applications. The transformation of a series of functionalities such as olefins, halides and tosylates into thiols is accomplished utilizing three facile routes. The utilization of these novel thiols in the formation of gold cluster micelles results in an excellent sensor that responds electrically, when exposed to a variety of vapors including organic solvents. A series of electrical response experiments was performed to evaluate each of the functionalized thiols to obtain a correlation and to maximize response to the molecules of interest. Additional efforts are underway to incorporate these sensors into a variety of real time detection devices. Synthetic challenges, sensitivity and application of these materials are discussed in detail.

**GUANINE-7-METHYLTRANSFERASE: ACTIVE SITE LABELING WITH RNA AND DNA.** Amber R. Bonham & Thomas O. Sitz, Dept. of Biochemistry, Virginia Tech, Blacksburg, VA 24061. The 5'-cap structure of eucaryotic mRNAs are modified through methylation by the enzyme guanine-7-methyltransferase (GMT). Without the methylation on the guanine base the mRNA does not function and therefore the mRNA is not translated into protein. We have been able to label the active site of this enzyme by cross-linking  $^{32}\text{P}$ -labeled DNA and RNA to the GMT with short wavelength UV light. We wanted to determine if the active site of GMT can discriminate between labeled DNA and RNA. We used a DNA oligo 11 deoxynucleotides long (11mer) and an RNA oligo 11 nucleotides in length (11mer) in the experiments. The DNA and RNA used are of the same sequence with the only differences being that DNA contains thymine while RNA contains uracil and DNA is composed of deoxyribose while RNA is made of ribose. The 5'-ends of the 11mer DNA and 11mer RNA were labeled with the enzyme polynucleotide kinase and  $\gamma$ - $^{32}\text{P}$ -ATP. With the use of MALDI-TOF mass spectrometry we were able to verify that the DNA and RNA were the same nucleotide length. These nucleic acids were bound and cross-linked to the GMT enzyme and analyzed on an SDS polyacrylamide gel. The enzyme guanine-7-methyltransferase binds DNA and RNA equally.

**A COMPARISON OF SUBUNIT STRUCTURE OF THE GUANINE-7-METHYLTRANSFERASE IN MOUSE AND HUMAN.** Heidi L. Miller & Thomas O. Sitz, Dept. of Biochemistry, Virginia Tech, Blacksburg, VA 24061. The methylation of the 5'-cap structure in eucaryotic mRNA is important in order to have a functional mRNA. Earlier research from this laboratory determined that the mouse guanine-7-methyltransferase (GMT) had a subunit size of 46,000 daltons on SDS-PAGE. Recent analysis of the cDNA sequences of mouse and human has predicted that the mouse and human GMT enzyme have molecular weights of 53,000. We wanted to determine the native molecular weights of the mouse and human GMT. Using gel exclusion column chromatography on an FPLC Superose 6 column, we found that the mouse GMT has a molecular weight of 97,000–120,000 daltons (depending on the fraction size collected). These results suggest that the mouse enzyme functions as a dimer. When compared with human GMT isolated from HOS cells, there were two peaks of activity. One peak was 142,000 daltons, and the second was 49,000 daltons. Human GMT can be found in both a monomer and dimer form. When human GMT was fused with glutathione transferase and expressed in *E. coli*, the molecular weight was found to be 180,000, which is most likely a dimer consisting of two subunits. Therefore, the mouse GMT is only active in the dimer form, while human GMT is active in both monomer and dimer forms.

**HIGH RESOLUTION INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETER (HR-ICP-MS): A POWERFUL TECHNIQUE FOR ULTRA-TRACE ELEMENTAL AND ISOTOPE RATIO ANALYSES.** Zhongxing Chen, Laboratory for Isotope and Trace Element Research, Department of Chemistry and Biochemistry, Old Dominion University, Norfolk, VA23529 & Cynthia M. Jones, Center for Quantitative Fisheries Ecology, Old Dominion University, Norfolk, VA23529. Since its commercial introduction in the early 1980s, inductively coupled plasma mass spectrometry (ICP-MS) has been widely used as a multi-element analytical technique for trace elemental determinations and isotope ratio measurements in various disciplines. However, limited sensitivity and low mass resolution of traditional quadrupole ICP-MS cannot meet two growing analytical demands, *i.e.*, lower detection limits and resolution of interferences. Recently installed at the Laboratory of Isotope and Trace Element Research of Old Dominion University, the new Finnigan MAT high resolution magnetic sector ELEMENT 2 high resolution (HR)-ICP-MS provides scientists a powerful technique to perform ultra-trace elemental and isotopic analyses. With sensitivity higher than 1.0 billion counts per second (cps) per ppm and dark noise less than 0.2 cps, elemental concentrations down to ppt (even ppq for some elements) levels can be measured. The determination of elements such as Fe, V, As, and Se has been problematic with conventional quadrupole ICP-MS because of the limited mass spectral resolution of that instrument. However, the high resolution capability of our HR-ICP-MS allows most of these elements to be distinguished from the interfering masses. A UV laser ablation (LA) system coupled with the HR-ICP-MS provides *in-situ* determinations of a wide range of trace element and isotope composition in solid. In this study, we present the performance of our HR-ICP-

MS and its application to ultra-trace elemental concentration determinations and isotope ratio measurements in marine, geological, environmental, biological, and nuclear samples.

**PHYTOESTROGENS IN BEER AND WINE.** Roy L. Williams & Mark Elliott, Old Dominion Univ, Enological Research Facility, Dept. of Chem./Biochem., Old Dominion Univ., Norfolk, Va. 23529. This laboratory has been known for its research related to wine and health and the study of the phytochemical composition of wine, grape seeds and more recently soy products. The positive health benefits associated with moderate, responsible consumption of wine has been related to the levels of various phytochemicals including the isoflavonoids and stilbene phytoestrogens in the wine. Earlier studies have shown that the levels of these phytochemicals can be enhanced by fermentation of wine on the appropriate grape seeds. We have now adapted this seed enhancement approach to the production of a phytochemically enhanced beer. Soybeans are known to contain high levels of certain phytochemicals including genistein, daidzein and trans-resveratrol (TR). We have now produced a soy-enhanced beer, which is rich in these phytochemicals, by the addition of a soybean extract during the fermentation process. The resulting beer is exceptionally high in genistein, daidzein and TR and constitutes a beer with enhanced positive health factors compared to other commercial beers. The method of production and high-pressure liquid chromatographic analysis of this soy-enhanced beer will be discussed.

**NATURAL ENDOCRINE DISRUPTERS IN DRINKING WATER.** Joshua Richey & Roy L. Williams, Dept. of Chem./Biochem., Old Dominion Univ., Norfolk, Va. 23529. Endocrine disruptors (EDCs) are organic compounds, which can effectively compete with natural estrogens for the estrogen receptor and cause some degree of activity at this receptor. The presence of a variety of synthetic organic EDCs have now been observed in drinking water and their presence is of considerable significance with regard to potential public health effects. Natural EDCs such as the phytoestrogens genistein, daidzein and trans-resveratrol (TR) have not been detected in drinking water supplies to date but their presence in certain types of raw waters is quite likely. The presence of these natural compounds could be of some importance to the drinking water industry and the detection of these compounds in natural waters would be an important research topic. This paper will discuss the types of compounds that might be characterized as natural EDCs and suggest ways of effectively isolating and identifying these compounds. Although EDCs are normally thought of as having a negative effect on the environment and as a possible health hazard, this paper will project a positive side to these natural EDCs.

**SYNTHESIS AND CHARACTERIZATION OF NEW TRANSITION METAL PHOSHPATES AND PHOSHPONATES.** Zeric Hulvey, William R. Gemmill & Barbara A. Reisner, Dept. of Chem., James Madison University, Harrisonburg, VA 22807. Open framework phosphate-based materials are of great interest because of their potential applications in fields ranging from separations and molecular recognition to catalysis. In an effort to synthesize new open-framework compounds containing transition metals, several strategies have been employed. Using these strategies, two new 2-D phosphate-based framework materials have been synthesized:  $[\text{HOCH}_2\text{CH}_2\text{NH}_3][\text{Co}(\text{PO}_4)]$  and  $\text{Zn}(\text{O}_3\text{PCH}_2\text{CH}_2\text{CH}_2\text{NH}_3) \cdot \text{H}_2\text{O}$ .  $[\text{HOCH}_2\text{CH}_2\text{NH}_3][\text{Co}(\text{PO}_4)]$  crystallizes in the orthorhombic space group  $Pca2_1$  ( $a = 7.6400(15) \text{ \AA}$ ,  $b = 13.380(3) \text{ \AA}$ ,  $c = 6.7467(13) \text{ \AA}$ ). It consists of inorganic cobalt phosphate layers that are separated by protonated ethanolamine ions. These ions are hydrogen bonded to the layers in a bidentate fashion.  $\text{Zn}(\text{O}_3\text{PCH}_2\text{CH}_2\text{CH}_2\text{NH}_3) \cdot \text{H}_2\text{O}$  crystallizes in the monoclinic space group  $P2_1/c$  ( $a = 9.0895(3) \text{ \AA}$ ,  $b = 5.0005(2) \text{ \AA}$ ,  $c = 6.7467(13) \text{ \AA}$ ,  $\beta = 3.337(3)^\circ$ ). It has a unique structure for a 2-D metal phosphonate; it contains a hybrid organic-inorganic layer. The layers contain 1-D  $\text{Zn}_2\text{P}_2$  ladders that are connected to each other by the amino end of the phosphonate group. The layers are held together framework water that is hydrogen bound to the layers.



A SYNTHETIC APPROACH TO 1-SUBSTITUTED  $\gamma$ -CARBOLINES. W. M. Stalick, C. A. Roberts, J. H. Wynne & S. Boson, Department of Chemistry, George Mason University, Fairfax, VA 22030-4444. Despite the increasing interests in tryptamines and their carbazole derivatives, pyrido[3,4-b]indoles, very little is known about their counterparts, the corresponding [4,3-b] analogues. This is primarily due to the lack of availability of such compounds as well as the inability to selectively introduce functionalities into these derivatives. We report two novel methods for their formation. The first proceeds from a 3-bromo-1-silyl protected indole which is condensed with a variety of N-tosylaldehydes. The second involves the acylation of indole followed by reductive amination; both methods afford a different variety of 3-aminomethyl derivatives. In either case subsequent intramolecular cyclization, followed by aromatization, in a one-pot process, affords the desired fully aromatic carbazole. This approach allows for control in the introduction of various substituents into the 1-position as desired.

BLACK GOO: A FUNGAL DISEASE IN GRAPEVINES. David McGinnis & Roy L. Williams, Old Dominion Univ. Enological Research Facility, Dept. of Chem./Biochem., Old Dominion Univ., Norfolk, Va. 23529. Over the past ten years many wine producing countries have described a common fungal disease that has come to be known simply as Black Goo. The source of this devastating disease is the fungus known as *Phaeoccremonium chlamydosporium*. This fungus infects the vine and causes a degradation of the vascular system and the subsequent development of a black gooe substance in the xylem of the plant. The nature and source of this Black Goo is still unknown and is of considerable importance to the wine industry. This laboratory has now been able to culture this fungus from infected vines from a Virginia vineyard and we are attempting to determine the chemical nature of the Black Goo substance that appears in the xylem. We are using various analytical methods including infrared analysis, high-pressure liquid chromatography and GC/MS to help characterize this material and to determine its origin in the plant. The nature and appearance of the Black Goo component appears to be associated with the synthesis and release of a natural antifungal phytoalexin known as trans-resveratrol.

THE DESIGN, SYNTHESIS AND EVALUATION OF NOVEL CALCIUM CHANNEL BLOCKERS IN HUMAN PLATELETS. Beth Dovel<sup>1</sup> & Roy L. Williams<sup>1</sup>, <sup>1</sup>Dept. of Chem./Biochem., Old Dominion Univ., Norfolk, Va. 23529 and Yuliya Dobrydyneva<sup>2</sup> & Peter Blackmore<sup>2</sup>, <sup>2</sup>Dept. of Physiological Sciences, Eastern Virginia Medical School, Norfolk, Va. 23508. 2-Aminoethoxydiphenyl borate (2APB) has been shown to be an effective inhibitor of store-operated calcium channels (SOCC) in human platelets. This inhibition of calcium influx in human platelets is important with regards to the potential of this compound as a cardiovascular agent and the treatment of heart disease and the aggregation of platelets in the blood stream. A number of structurally related compounds have been designed based on the proposed mechanism of action of this drug and these compounds have now been synthesized and characterized in this laboratory. The scope of the synthetic approach to the design of a more effective calcium channel blocker will be discussed together with the method of synthesis and analytical features of these novel compounds. Nuclear magnetic resonance data will be presented that confirms the presence of a five membered ring in many of the simpler 2APB analogs. Structure activity relations of the various 2APB analogs will be discussed in an effort to rationalize the mechanism of action of these agents in human platelets.

THEORETICAL STUDIES OF PRECURSOR AMINES OF NOVEL ANTIMICROBIAL QUATERNARY AMMONIUM SALTS. Juan C. Jimenez, Annick Charlotte, Sabrina M. Aponte, & Edmund Moses N. Ndip, Dept. of Chemistry, Hampton University, Hampton, VA 23668. The focus of our study is to determine structure – property and structure – activity relationships for a new class of quaternary ammonium salts (QAS). This paper presents results presents results of conformational analysis, optimized geometries and some electronic properties of series of a number of model precursor amines. The anomeric effect is also presented. Molecular simulations (modeling) were done using semi-empirical (AM1, AM1-SM2, PM3) and Monte Carlo routines contained in



MACROMODEL. The calculations suggest that the hydrogen -- bonded structure is the preferred conformation for precursor amines, and simple QAS instead of the hemicholinium. Electrostatic potential plots have been obtained. These are to be used to determine active sites for interaction between drug molecule and a potential receptor site.

THE HYDROPATHIC ANALYSIS OF DNA INTERCALATORS TO DESIGN HIGHLY SEQUENCE-SPECIFIC COMPOUNDS. Derek J. Cashman<sup>1</sup>, Glen E. Kellogg<sup>1</sup>, & J. Neel Scarsdale<sup>2</sup>, Dept. of Med. Chem.<sup>1</sup>, Virginia Commonwealth Univ., Richmond, Va., 23219-1540., & Dept. of Biochem. & Mol. Biophysics<sup>2</sup>, Virginia Commonwealth Univ., Richmond, Va., 23219-1540. Molecular models of the potent anticancer compound doxorubicin, 59 analogs, and their intercalation complexes in 6 unique DNA quartet sequences were minimized using molecular mechanics techniques. The molecular modeling program HINT (Hydropathic INTERactions) was then used to calculate quantitative binding scores of each complex. The difference in the HINT interaction scores in all six sequences was then analyzed and compared. Methods to analyze these interaction scores and compare them to the structure of the compounds will be discussed. In addition to a total HINT score for the binding of the drug with DNA, individual HINT scores can be calculated between the drug and the four individual base pairs that are in the vicinity of the intercalation site, to analyze the individual parts of the drug molecule that are contributing to selectivity. Further correlations between HINT scores and experimental  $\Delta G$  will also be presented for some compounds.

THE ROLE OF IONIZATION STATE IN HINT CALCULATIONS OF BINDING FREE ENERGY FOR INFLUENZA VIRUS NEURAMINIDASE-INHIBITOR COMPLEXES. M. Fornabaio<sup>1,2</sup>, G. E. Kellogg<sup>1</sup>, A. Mozzarelli<sup>2</sup>, P. Cozzini<sup>2</sup> & D. J. Abraham<sup>1</sup>, <sup>1</sup>Dept. of Medicinal Chemistry, Virginia Commonwealth University, Richmond, VA 23298 and <sup>2</sup>Dept. of Biochemistry and Molecular Biology, University of Parma, Italy. The prediction of binding affinity between protein and ligands is a key problem for computational biochemistry and drug discovery. HINT (Hydropathic INTERaction) is a software model based on experimentally determined solvent partitioning data between water and 1-octanol ( $\text{LogP}_{o/w}$ ) to obtain an empirical model of biomolecular association. As  $\text{LogP}_{o/w}$  can be directly correlated with free energy, HINT's peculiarity is that it can evaluate not only the enthalpic contributions but also the entropic contributions to ligand binding. This software can predict, with reasonable accuracy, the free energy of ligand binding. The present work is focused on the calculation of binding free energy for Influenza Virus Neuraminidase-inhibitor complexes, highlighting the role of the ionization state of ligands and of protein active site residues (Asp or Glu) for model building. Attention is paid to the relationship between the ionization state and the pH conditions under which the binding measurements are made and the correlation between experimental free energy and HINT scores is shown.

NUCLEOPHILIC SUBSTITUTION MECHANISMS – SCRATCHING THE SURFACE. Charles M. Bump, Department of Chemistry, Hampton University, Hampton, VA 23668, The purpose of this work is to examine nucleophilic substitution reactions in order to better understand and differentiate between  $S_N1$  and  $S_N2$  type reaction mechanisms. Using the public domain release of MOPAC 6.0 ported to the PC, semi-empirical (AM1) potential energy surfaces for carbon to nucleophile distances of 10.0 to 1.5 angstroms and carbon to leaving group distances of 1.5 to 10.0 angstroms were generated for the (gas phase) reactions of n-butyl bromide, sec-butyl bromide, and tert-butyl bromide with chloride ion. In  $S_N1$  reactions, the leaving group departs while the incoming nucleophile is further away from the reaction site than it does in an  $S_N2$  reaction. There is little difference between reaction mechanisms in terms of charge associated with the incoming nucleophile, the carbon at the reaction site, and the leaving group. While there are regions of relatively high stability that are outside the reaction path, the activation energy required to assume those conformations is prohibitive.

### Computer Science (No Abstracts Submitted)

### Education

**DISCOVERING WOMEN IN THE BIOLOGICAL SCIENCES: A LABORATORY COURSE FOR NON-SCIENCE MAJORS.** Elsa Q. Falls, Dept. of Biol., Randolph-Macon College, Ashland, VA 23005. Traditionally, women have not been well-represented in the sciences in the United States. They have served historically as assistants to male scientist relatives, but their names and specific contributions have been largely hidden. Dating back to Charles Darwin, cultural biases have indicated that women are inferior to men. Currently, women make up over 50% of the country's workforce but only about 16% of employed scientists and engineers. Explanations for this disparity have included gender differences in intellectual abilities and differing experiences in the sciences. There continue to be barriers to women's participation in science and segregation among institutions and scientific fields. The current on-going investigation involves collection of data on non-science majors' perceptions and knowledge of contemporary and historical science and scientists. It was hypothesized that a course for non-science majors highlighting historical and contemporary women biologists and their contributions would change student attitudes toward science and scientists. A collegiate requirement laboratory course was developed and is being offered for the first time during spring semester 2002. Pre- and post-course surveys of these students, as well as students in other non-science major courses are being conducted to determine if attitudes can be changed.

**DEMONSTRATING THAT HABITAT STRUCTURE FACILITATES COEXISTENCE OF PREY AND PREDATOR.** Tracey R. Embrey & Timothy W. Stewart, Department of Natural Sciences, Longwood College, Farmville, VA 23909. Habitat structure, defined as physical objects in an environment that provide habitat, is important in ecosystems because it regulates organism abundance and species diversity. We designed a laboratory experiment to show students that habitat structure increases prey survivorship by providing refuges from predation. In the experiment, goldfish held in aquariums forage on two species of invertebrate prey in both the absence (no-structure treatment; n = 5 replicates) and presence (structure treatment; n = 5 replicates) of habitat structure created by stones and ceramic tiles. Results from two-sample t tests generally show that a significantly greater number of bottom-dwelling *Gammarus* (i.e., amphipods) survive in the structure treatment than in the no-structure treatment. In contrast, *Daphnia* are open-water organisms that do not use the structure we provide to them, and *Daphnia* survivorship in the two treatments does not differ. Results demonstrate that by creating refuges, habitat structure increases the likelihood of predator-prey coexistence. Additionally, diverse forms of habitat structure are required to provide refuges for multiple prey species, and to maintain high species diversity in ecosystems.

**GAUGING SCIENCE LITERACY THROUGH THE ANALYSIS OF STUDENT WRITING: RUBRIC DEVELOPMENT USING UNDERGRADUATE NON-SCIENCE MAJORS.** Woody McKenzie & Clancy Leahy, Lynchburg College, Lynchburg, VA 24501. A rubric of student writing to rate attributes that define science literacy was developed using primary trait analysis. In this pilot study, one evaluator rated graded written exams. Students chose from four different current topics (threat of biowarfare, alternative fuels legislation, mammograms for detection of breast cancer, and genetic engineering as a cure for diabetes). Each topic included a set of specific prompt questions to guide students. The rubric used Likert type rankings to assess the student's ability in: 1.) posing the question, 2.) research acquisition, 3.) research evaluation, and 4.) use of research. Of twenty-nine papers evaluated, four were rated expert. Eleven showed serious inadequacies in ability to research and make well-reasoned decisions on complex issues requiring information gathering and filtering in the scientific realm. In general, students were able to ask relevant questions, but relied heavily upon internet sources for evidence. In many cases, some "evidence" did not directly relate to the question

posed. Interestingly, students researching biowarfare displayed the lowest level of science literacy. More examples of student writing need to be evaluated. More evaluators need to rate these to establish inter-rater reliability. More piloting needs to be done with students receiving fewer prompts

FIRST YEAR STUDENTS' SATISFACTION WITH COLLEGE AND SENSE OF BELONGING: A COMPARISON OF LEARNING COMMUNITY AND NON-LEARNING COMMUNITY STUDENTS. Jennifer Ann Morrow, J. Worth Pickering, & Sandra M. Waters, Old Dominion University, Norfolk, VA 23529. Old Dominion University began offering Learning Communities in the Fall of 1996 as a way to address the increasing problems of students in academic difficulty and attrition rates. Learning Communities at ODU are made up of a cohort of approximately 25 students who are enrolled in three linked general education courses. Early research on Learning Communities at ODU, showed that Learning Community students had more positive relations with faculty members and fellow students. Learning Communities also showed a positive impact on academic difficulty, academic success, and retention rates. In the Fall of 2001, Learning Community students reported significantly higher levels of seeing connections between their classes, making friends in classes, and intention to return for their sophomore year. Learning Community students also reported significantly higher levels of perceived peer support and significantly lower levels of perceived isolation. Next Fall, approximately 50% of entering First Year students will be enrolled in either Curricular or Residential Learning Communities.

DISCOVERING SCIENCE THROUGH MICROBIOLOGY: BIOLOGY MAJORS, NON-SCIENCE STUDENTS, AND HIGH SCHOOL STUDENTS. D. L. Wohl<sup>1</sup>, P. B. Lessem<sup>1</sup>, & J. B. Reed<sup>2</sup>, University of Richmond, Richmond, VA 23173 and <sup>2</sup>the Greater Richmond Area Higher Education Consortium (GRAHEC), 4901 Fitzhugh Ave. Suite 301, Richmond, VA 23230. We are offering experiential learning opportunities to three different populations of students including University of Richmond undergraduates (biology and non-science students) and high school students enrolled in GRAHEC programs. To maximize student learning and efficiency in presentation, we have developed one general course design that can be delivered to our three targeted populations. Both the non-science student's course (*Unseen Life*) and the biology major's course (*Microbiology*) will be offered in the spring semester resulting in similar preparations. Similar in design and subject, *Microbes: Life's Small Beginnings (Microbes)* can then be offered that summer to high school students. All three courses will use microbes isolated from area waters for further analysis. Investigations will focus on antibiotic resistance in environmental isolates. This project is supported by the NSF division of Undergraduate Education : Course, Curriculum and Laboratory Investigation.

### Environmental Science

CHARACTERIZATION OF *Pseudomonas fluorescens* STRAIN 29L AND DEGRADATION OF PYRENE AS SOLE CARBON AND ENERGY SOURCE. R. J. Ganguli, J. Ottke & C. D. Litchfield, Dept. of Environmental Science & Policy, George Mason Univ., Manassas, VA. A pyrene degrading bacterium, isolated from a creosote-contaminated site, using Bushnell-Haas medium supplemented with 0.3 gm/L medium each of naphthalene, phenanthrene, and pyrene. It was identified as *Pseudomonas fluorescens* Strain 29L by BIOLOG<sup>TM</sup> and further confirmed by 16S rDNA analysis. Strain 29L was able to grow in a medium containing NH<sub>4</sub>Cl as the nitrogen source supplemented with 0.001% yeast extract and up to 300mg/L pyrene as the sole source of carbon and energy. CO<sub>2</sub> respirometric studies using biometer flasks revealed that nearly 20% of the 50mg/L pyrene added was respired as CO<sub>2</sub>. Phenanthrene, anthracene, chrysene, salicylic acid, and phthalic acid also support the growth of this bacterium. Mineralization of <sup>14</sup>C-labeled pyrene to <sup>14</sup>CO<sub>2</sub> indicated that pyrene did serve as sole carbon and energy source. Strain 29L was subjected to various curing agents to determine whether this pyrene-degrading capability is plasmid-borne. Pyrene is one of the priority

pollutants listed by USEPA. Studies on pyrene degradation will help in devising better technologies for the biodestruction of such hazardous chemicals.

**METAMORPHOSIS-DEPENDANT LEAD SENSITIVITY IN AMERICAN BULLFROG LARVAE.** James A. Wise, Dept. of Biol. Sci., Hampton Univ, Hampton, Va 23668. Lead toxicity is still a major health problem in the U.S., especially in children. Children exposed to low lead levels show a variety of developmental defects. In spite of these data, the lowest level of lead exposure that a child can safely be exposed to has not yet been established. Our laboratory has developed an American Bullfrog (*Rana catesbeiana*) larval model to investigate the effects of chronic, low level lead exposure on their physiology. These data from this developmental amphibian model will be used to develop a risk assessment model for low-level lead exposure in developing human beings. Initial studies showed differential sensitivity ( $LC_{50}$ ) of the larvae to high-level lead exposure in a 24 hr, Acute Toxicity Test (ATT) according to developmental stage. In this study, we developed a 7-day, Chronic Toxicity Test (CTT) to more accurately model environmental lead exposure. We found that tadpoles exposed to lead in the CTT were likewise differentially sensitive according to developmental stage, and that the larvae in this test were sensitive to levels of lead ( $>10$  ppm) an order of magnitude lower than that seen in the ATT. These levels are in the range of the lowest acceptable lead exposure are recommended for human being (approximately 10 ppm). Thus, the CTT model will serve as a springboard for further investigations into the effects of lead exposure on developmental physiological processes.

**USE OF PULSED-FIELD GEL ELECTROPHORESIS FOR TYPING FECAL CONTAMINATION IN RECREATION WATERS.** Matthew R. Ettinger<sup>1,2</sup> & Denise A. Pettit<sup>1,2</sup>, <sup>1</sup>Department of Microbiology and Immunology, Virginia Commonwealth University, Richmond, VA 23298 and <sup>2</sup>Division of Consolidated Laboratory Services, Richmond, VA 23219. The introduction of fecal bacteria into environmental waters can increase the incidence of infectious diseases in populations recreating in contaminated areas. Polluted watersheds are identified using standard environmental microbiological techniques to quantitate a variety of indicator organisms, including *Escherichia coli* (*E. coli*). Restoration plans designed to bring impaired waters back into compliance require the identification of point and nonpoint sources of fecal pollution in the watershed. This process is often hampered by difficulties in correctly identifying nonpoint sources of pollution. To facilitate the identification of nonpoint sources of contamination, the molecular typing technology of Pulsed-Field Gel Electrophoresis (PFGE) has been employed. Isolates of 820 *E. coli* derived from the fecal material of 28 different source groups were lysed and DNA was digested with Xba I. DNA fragments were separated using PFGE and banding patterns were analyzed using Jaccard and Dice analysis. Isolates obtained from individual animals of the same species did not cluster. Therefore, new approaches to the analysis of the banding patterns are currently being investigated to determine if PFGE is a viable means of identifying nonpoint source pollution in recreational waters.

**FEEDING BEHAVIORS OF BOTTLENOSE DOLPHINS IN THE ELIZABETH RIVER, VA.** J. Stepp, K. Foss, and J. Reed, Dept. of Biology, Chemistry, and Environmental Science, Christopher Newport University, Newport News, VA 2360. The purpose of this study is to describe the utilization of the Elizabeth River by bottlenose dolphins. The Elizabeth River, a tributary of the Chesapeake Bay, has been characterized as the third most polluted river on the east coast. In this paper, I describe the feeding behaviors of bottlenose dolphins. Dolphins are generalist feeders and capture a variety of prey ranging from fish to squid. During 2001, a total of 59 cruises, lasting an average of three hours, were conducted from January to October. Dolphins were encountered on 28 occasions. Group sizes ranged from one adult to 80 adults. Dolphins were observed engaging in probable feeding activities during 18 encounters. During periods of probable feeding, dolphins were observed moving closer to shore. Indicators of probable feeding were a strong fish smell in the immediate area, deep diving, and birds feeding in the immediate area. Probable feeding behavior was also indicated by fish swirls and bouts of fish tossing. On several occasions, groups that were spread

out were observed moving closer together, then spreading out again. These encounters were said to include bouts of probable feeding activity since a strong fish smell was present. Fish were detected on the sounder. A pattern of probable feeding activity with respect to time of day was seen. Activity increased during the morning and decreased during the afternoon. Probable feeding activity appeared to increase with water temperature. Sampling in areas of probable feeding activity indicated that spot and croaker were potential prey.

**BASELINE WATER QUALITY STUDY OF ENGLAND RUN AND AN UNNAMED TRIBUTARY TO THE RAPPAHANNOCK RIVER.** Emma B. Law<sup>1</sup>, M.L. Bass<sup>1</sup> & M.L. Daniel<sup>2</sup>, <sup>1</sup>Dept of Environmental Science & Geology, Mary Washington College, Fredericksburg, VA 22401 and <sup>2</sup>OWML of Dept of Civil & Environmental Engineering of Virginia Tech, Blacksburg, VA. Surface water quality is influential and must receive consideration as human development continues to encroach upon our natural waterways. Celebrate Virginia is a proposed 1,500 acre development for Stafford County, Virginia. The development will directly impact England Run and an Unnamed Tributary. This study examined physiochemical measures monthly from November 2000 to December 2001 at seven strategic stations using EPA approved methods. Depth, flow rate, water temperature, pH, dissolved oxygen, biochemical oxygen demand, conductivity, total alkalinity, total hardness, total phosphorous, total nitrogen, total dissolved solids, and total and fecal coliforms were monitored and used to characterize the tributaries' current water quality prior to construction. Continued monitoring will allow impacts of Celebrate Virginia to be quantitatively identified. Currently, England Run and the Unnamed Tributary appear to be typical Piedmont streams with all parameters in an expected range except for coliform counts, which were elevated.

**THE SIGNATURE OF GLOBAL WARMING IN THE CHESAPEAKE BAY, VA.** T.C. Mosca III, Rappahannock Community College & W. Coles, Govt. of the Virgin Islands. Water temperature data collected at Virginia Institute of Marine Science at Gloucester Point, Va. from 1954 until the present were analyzed for trend. Water temperature is a sum of many functions, some periodic and some not. By collapsing the data to annual summer and winter means, the long-term trend was exposed. The trend was established to exist in the entire Virginia portion of the Chesapeake Bay, by regression against similar means of data collected by the VIMS trawl survey. The long-term trend indicates increasing temperatures, with a recorded change of 1.5 °C over the period of record. An interesting spatial/temporal pattern was also noted.

**OBSERVATIONS ON THE OCCURRENCE OF *CLADOSPORIUM*, *PENICILLIUM* AND *STACHYBOTRYS* SPECIES IN AN INDOOR ENVIRONMENT, EXHIBITING PREFERENCES TO DIFFERENT BUILDING MATERIALS.** Bharati Lakshmi, Environmental Testing and Monitoring Services, 2425 Bowland Parkway, Virginia Beach, VA 23454. Molds occur indoors due to moist building materials like insulation, paper and wood causing health problems like allergy, infection, irritation and toxicity at high concentrations. During renovation of an abandoned school building extensive mold growth was observed on stairwells and walls. Studies were conducted to identify the building materials affected by mold and analyze the materials for mold identification (genus level) and concentration (Colony Forming Units per gram (CFU/g)). Large quantities of mold spores were observed on textured plaster ceiling, cinderblock wall, two-coat plaster wall and gypsum and paper ceiling. *Cladosporium* sp. (2000 - 200,000 CFU/g) was identified on all the four building materials. However, *Penicillium* sp. (3000 - 50,000 CFU/g) occurred on all except textured plaster ceiling and *Stachybotrys* sp. (3000 - 60,000 CFU/g) occurred exclusively on gypsum and paper ceiling. *Stachybotrys* exhibited very high preference for gypsum and paper based material, *Penicillium* showed medium preference and *Cladosporium* exhibited least preference.

RELATIONSHIP OF FLUORIDES TO STRENGTH MEASUREMENTS IN MULE DEER ANTLERS. Mary T. Lovegreen, P. F. Scanlon, & J. H. Wilson. Depts. of Fisheries and Wildlife Sciences and Biological Systems Engineering, Virginia Tech, Blacksburg, VA 24061-0321. As "hard" tissues, deer antlers have been found to share many characteristics with bones and teeth in humans. Antlers represent a deciduous source of a substitute for bones and teeth in studies of the latter two tissues. Broken antlers have been found on deer in areas with high concentrations of natural fluoride. This project endeavored to relate concentrations of fluorides to a strength measurement of antlers of mule deer (*Odocoileus hemionus*). Antlers from two sources were available for the project: Intact antlers on hunter-killed or road-killed mule deer from Colorado Springs area, CO, and shed antlers from mule deer from Pinyon Canyon, CO. Antler sections of 2.5cm length were sawed from antler tines and the cross-sectional area of each was measured. The segments were subjected to a strength test (compression to failure) using an MTS machine. The test generated a parameter 'peak load' which when divided by cross-sectional area gave a parameter 'peak stress'. Fluoride concentrations in solutions of antler tissues were measured with an Orion portable Ion Specific Electrode attached to a pH meter with millivolt reading capabilities. Peak stress was plotted against fluoride and regressions were run. Fluoride concentrations were higher in antlers of deer from the Colorado Springs area than in deer from the Pinyon Canyon area. Measurements of antler strength were somewhat variable and regressions of strength measurements on fluoride concentrations were not particularly illustrative in determining relationships between the two variables. Fluoride seems to influence antler strength in different ways at different concentrations. Initially, fluoride tends to increase antler strength, but at higher concentrations tends to increase brittleness.

AVAILABILITY OF DEN TREES FOR BLACK BEARS IN FORESTS WITH DIFFERENT MANAGEMENT STRATEGIES. Gyasi A. Quince, P.F. Scanlon & M. R. Vaughan, Dept. Fisheries and Wildlife Sciences, Virginia Tech., Blacksburg, VA 24061-0321. Availability of den trees is of great importance to hibernation by black bears, *Ursus americanus*, in the Appalachian Mountains. This report compares availability of den trees on National Forest lands and on industrial forests of Westvaco Corporation in Virginia and West Virginia. Five study areas were chosen: 3 National Forest (2 in Virginia and 1 in West Virginia) and 2 industrial forest sites (1 each in Virginia and West Virginia). Within each of the 5 locations stands with ages within the ranges < 50 years post-harvest, 51 to 100 years post-harvest, and 100+ years post-harvest were chosen. Five quadrats, each 40m x 400m were randomly selected within each age-group for detailed examination of the presence of den trees. Potential den trees, specifically those of DBH 75cm or greater, were recorded and tested for soundness. Unsound trees were considered to have cavities and these were checked to evaluate the quality of the cavity. Those cavities with entries equal to or larger than 43cm high and 24cm wide were considered suitable for denning. There were no 100+ years stands on the Virginia Westvaco site so only 70 quadrats were available for sampling. Potential den trees were more common in 50-100 and 100+ quadrats. Unsound trees were relatively few (total =27). Chi-square analyses indicated that the proportion of potential den trees was independent of location (VA vs. WV) and of forest management style (Westvaco vs. National Forest). Den trees were too few to draw meaningful conclusions. We appreciate support from, Westvaco Corporation, Virginia Tech, Dept. Fisheries and Wildlife Sciences and the MAOP Program, Virginia Department of Game and Inland Fisheries, U. S. Forest Service, and USGS-Biological Resources Division.

ACCEPTABILITY OF CLAY TARGET FRAGMENTS AS GRIT BY CAPTIVE JAPANESE QUAIL. Gabriela R. Gonzalez, P. F. Scanlon, K. E. Webb, Jr., M. R. Vaughan, & J. R. Craig. Depts. Fisheries and Wildlife Sciences, Animal and Poultry Sciences, and Geological Sciences Virginia Tech, Blacksburg, VA 24061. Lead toxicosis in wildlife on shooting ranges has had considerable study. However, less is known on the use by, and effect of, clay targets on local and migratory wildlife, particularly avian species. Clay targets, used for shooting practice, are composed of "clay" (dolomitic limestone) and petroleum pitch, depending on the actual target brand. Bound within the pitch are polycyclic aromatic hydrocarbons (Baer et al. *Ecotoxicology* 4:385, 1995). In this study, 36 Japanese

quail (*Coturnix coturnix japonica*) were individually confined in 50 x 30 x 23 cm cages and offered crushed samples of clay targets and/or ground limestone as grit. The objective was to determine if the birds chose the target material as grit. The quail were fed a commercial birdseed diet ad libitum. Crushed targets (size range: 1.19 to 3.36mm) and limestone, of a comparable range of sizes, were offered in separate cups. Targets of two brands, Remington Blue Rock and Federal White Flyer, were offered, resulting in two colors, orange and yellow being available as options offered. Fragments with color were separated from basic black fragments. Each treatment consisted of four birds, two males and two females. Of the 32 quail offered targets, 19 consumed some of the target materials. However, of the 12 birds offered a limestone vs. target choice, 11 birds preferred limestone to the target. This experiment was preliminary and results warrant further investigation of the acceptability and effect of clay targets on birds. [Supported by the John Lee Pratt Animal Nutrition Foundation at Virginia Tech].

CHARACTERISTICS OF SPENT LEAD SHOT AND PARTICLES AT A SHOTGUN RANGE. James R. Craig<sup>1</sup>, David Edwards<sup>1</sup>, J. Donald Rimstidt<sup>1</sup>, Patrick F. Scanlon<sup>2</sup>, Thomas K. Collins<sup>3</sup>, Oliver Schabenberger<sup>4</sup>, & Jeffrey B. Birch<sup>4</sup>. 1) Geological Sciences, (2) Fisheries and Wildlife Sciences, and (4) Statistics, Virginia Tech, Blacksburg VA and (3) US Forest Service, Roanoke, VA. Systematic studies of spent lead shot and related metals at the public recreational shotgun range in the Jefferson National Forest just west of Blacksburg, VA have revealed that the areas impacted by shooting are much greater than the 0.7 acres cleared for the range and that the distribution of the lead is non-uniform. Shooting is either at stationary or airborne targets. Stationary target shooting concentrates lead at 25-30m from the shooting box where targets of considerable variety are set. The slight incline of the range and nearly horizontal trajectory of fire concentrates the lead in the central area over a relatively short range of distances. In contrast, when shooting at air-borne targets shotguns are fired at elevated angles and most of the lead pellets carry much greater distances. This study found a peak of lead concentration at 80-100m from the shooting box and a gradual decrease in concentration out to 300m. The size of lead particles dispersed on the shotgun range vary widely because of (1) the differences in shot sizes used and (2) abrasion of lead to form small grains and shards. Most shot falling to the ground retain nearly spherical shapes and are in the range 2-3mm. However, the discharge of shot from the shotgun barrel results in the generation and dispersal of fine particulate lead in areas close to the shooting box. In addition, the impact of lead pellets with targets and the ground at short distances also results in the abrasion of pellets and the release of fine lead particles in the range at 25-30m. The release of large quantities of fine particulate lead increases potential reactivity of lead in the environment and would make the recovery of lead more difficult.

## Geography

(No Abstracts Submitted)

## Geology

COMPLEX HYDROGEOLOGIC VARIABILITY AT SHIRLEY PLANTATION, CHARLES CITY COUNTY, VIRGINIA. G. R. Whittecar and R. B. Cole, Dept. Ocean Earth and Atmos. Sci., Old Dominion Univ., Norfolk, VA 23529. Significant changes in the thickness and permeability of Quaternary strata on fluvial and estuarine Coastal Plain terraces can occur over short lateral distances, marked by only subtle topographic signatures. A stratigraphic study done in support of a groundwater monitoring project revealed the importance of fluvial histories in limiting the extent of coarse-grained sediments beneath seemingly uniform terrace surfaces. Terraces underlain by the Pleistocene Tabb Formation are sliced by small valleys filled with fine-grained Holocene sediments that support perched water tables. These terrace surfaces may show little geomorphic expression of the filled valleys, even though the Holocene sediments may be as much as 10 meters thick. Hydraulic

heads in shallow wells placed in these valley fills respond quickly to rainfall events; deeper wells respond to seasonal changes in recharge. Additional stratigraphic variations at this study site come from eroded, partially buried remnants of older Pleistocene formations. The study area also lies over a former gravel quarry, backfilled with waste sediments from adjacent gravel operations. The permeability of mine wastes varies according to the degree of sorting inherent in the backfilling process (dumping vs. settling).

MINERALOGY, PETROGRAPHY, SCANNING ELECTRON MICROSCOPY AND BACKSCATTERED ELECTRON MICROSCOPY OF MIDDLE DEVONIAN SHALES FROM HIGHLAND COUNTY, SOUTHWESTERN VIRGINIA. P. S. Sethi, L. L. Combs & M. J. Woodard, Department of Geology, Radford Univ., VA 24142. Mineralogical analyses were conducted for a total of twenty-three samples spanning the laminated Millboro Shale and the bioturbated Needmore Shale. A total of 144 photomicrographs were obtained along with over 150 SEM and BSEM images. The Millboro Shale is characterized by parallel horizontal to parallel discontinuous stratification that is overprinted by discontinuities sub-parallel to the dominant lamination direction. SEM data show typical stepped domains and stepped card-house fabric reflecting inherent lamination at the micron level. The Needmore Shale samples show generally discontinuous original lamination with varying levels of bioturbation. SEM and BSEM data show typical disrupted clay fabric associated with micron-scale bioturbation. SEM and BSEM data also reveal differences in the morphology of the pyrite phases with the Millboro Shale samples containing fewer but larger vein structures of pyrite in comparison to the Needmore Shale. This study was funded in part by the Office of Vice-President for Academic Affairs at Radford Univ.

INVESTIGATION OF THE CONTROL OF TEMPERATURE ON MINERALOGY AND SOIL GEOCHEMISTRY OF TWO PLEISTOCENE SOIL PROFILES FROM SALTVILLE, VIRGINIA. P. S. Sethi, R. C. Whisonant, J. Surber, C. Boyd & V. Guilliams, Department of Geology, Radford Univ., VA 24142. Saltville, the Salt Capital of the Confederacy, produced over 200 million lbs. of salt during the Civil War. This study investigated the variations in soil color in close proximity to two of the iron furnaces used for the salt making operation. Soils just below the excavated furnace sites are deep red in color in contrast to the medium yellowish soil farther away from the furnaces. Detailed mineralogical and geochemical analyses indicated that the color variation is a result of a progressive change in the oxidation state of iron (ferrous to ferric ratio) caused by heat radiating from the furnaces. Laboratory experiments involving gradually heating the native yellow soil in a ceramic kiln under controlled climate conditions were successful in replicating such a heat effect on the oxidation state of iron. Changes observed in color of samples were very similar to those at the two archaeological sites in Saltville and lend support to the 'contact metamorphic' baking history of the underlying soils. This study was funded by a NASA grant to RCW (Contract # 961517).

### **Materials Science**

(No Abstracts Submitted)

### **Medical Science**

THE ANTINOCICEPTIVE EFFECT OF THC INVOLVES MODULATION OF ENDOGENOUS OPIOIDS. Melinda L. Cox & Sandra P. Welch, Dept. of Pharmacology, VCU, Richmond, Va 23298. Several lines of evidence have demonstrated interactions between cannabinoids and opioids. We addressed the hypothesis that THC-induced release of endogenous opioids results in antinociception in arthritic and normal (NA) rats. Using the Freund's Adjuvant-Induced Arthritis (FAA) test, male Sprague-Dawley rats were rendered arthritic in 19 days. THC (i.p.) and



morphine(s.c.) were found to be equally potent in both groups in the paw pressure test of antinociception. Morphine-induced antinociception, attenuated by naloxone in NA and FAA rats, was mediated by the mu opioid receptor. THC-induced antinociception, attenuated by SR141716A and naloxone in both groups, had a CB1 and opioid receptor component. A kappa opioid component was suggested by the attenuation with nor-BNI in FAA rats only. THC (i.p.) releases dynorphin A (dyn A) and leu-enkephalin (leu-enk) in CSF of NA rats, but tends to decrease release in FAA rats which tended to have higher levels after vehicle treatment. Dyn A interaction with the NMDA receptor may contribute to hyperalgesia in the FAA rat, and THC may induce antinociception by decreasing dyn A release. Thus, the interaction of dyn A with the kappa opioid receptor predominates, resulting in antinociception.

A COMPARISON OF THE BINDING OF THREE SERIES OF NICOTINIC LIGANDS. M.Lee<sup>1</sup>, M.Dukat<sup>1</sup>, L.Liao<sup>1</sup>, D. Flammia<sup>1</sup>, M. I. Damaj<sup>2</sup>, B. Martin<sup>2</sup> and R.A.Glennon<sup>1</sup>, <sup>1</sup>Department of Medicinal chemistry and <sup>2</sup>Department of Pharmacology and Toxicology, Virginia commonwealth University, Richmond, VA 23298. A total of 24 aryl-substituted analogs of nicotine and two related series of nicotinic ligands: aminomethylpyridines and ether analogs, were examined to determine if they bind at  $\alpha_4\beta_2$  nACh receptors in a common manner. A modest correlation ( $r=0.794$ ) was found between the affinities of the nicotine analogs and aminomethylpyridines, but little correlation ( $r = 0.117$ ) was found with ether analogs. However, a modest correlation ( $r = 0.783$ ) exists between the binding of aminomethylpyridines and ether analogs. It seems that the nicotine series and ether series compounds bind differently but that the aminopyridine series compounds share some intermediate binding similarity with both.

FE AND MN COMPETITION FOR *BACTEROIDES* APO SUPEROXIDE DISMUTASE *IN VIVO*. S.J Wise & E.M. Gregory, Department of Biochemistry, Virginia Tech, Blacksburg, VA. *Bacteroides fragilis* (ATCC 25285) synthesizes an aposuperoxide dismutase (SOD) that is active with either Fe or Mn at the active site. Under anaerobiosis, FeSOD was synthesized in anaerobic peptone-yeast-glucose medium (PYG) even with  $MnCl_2$  (1 mM). Treatment of the medium with metal-chelating resin (Chelex<sup>TM</sup>) diminished the Fe content from 20  $\mu M$  to 0.5  $\mu M$  and Mn content from 1  $\mu M$  to a value below the ICP detection limit. In media thus treated (c-PYG) but supplemented with 1-10  $\mu M$   $MnCl_2$ , *Bacteroides* synthesized an increasing amount of manganese superoxide dismutase (MnSOD). Cells grown in c-PYG medium supplemented with 10  $\mu M$  Mn synthesized approximately equal amounts of Fe- and Mn-SOD. In c-PYG supplemented with 10  $\mu M$   $MnCl_2$  and 1  $\mu M$   $Fe_2(NH_4)_2(SO_4)_2$ , *B. fragilis* synthesized 80% FeSOD, 20% MnSOD. The specific activity of superoxide dismutase was comparable in each cell extract. Under conditions of stringent iron restriction *Bacteroides fragilis* may maintain cellular SOD levels by using Mn and sparing Fe for other cellular functions. (Supported in part by a research scholarship from the John Lee Pratt Animal Nutrition Program)

EFFECT OF BUPROPION AND METABOLITES IN NICOTINE-TRAINED ANIMALS. Tatiana S. Bondareva, Mikhail L. Bondarev, Richard Young & Richard A. Glennon, Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, VA 23298. Bupropion is a weakly potent stimulant used both as an antidepressant and as a smoking cessation agent. Bupropion's mechanism of action is not well understood in both cases. We used a drug discrimination method to examine the effect of bupropion and its metabolites in rats trained to discriminate the effect of 0.6 mg/kg of (-)nicotine from saline. In tests of stimulus antagonism, bupropion failed to antagonize the stimulus effects of nicotine. However, in tests of stimulus generalization, bupropion substituted for nicotine. Unlike nicotine, stimulus effects produced by bupropion are not antagonized by mecamylamine. We tested two of the major metabolites of bupropion (+)-*threo*-2-tert-butylamino-1-(3-chlorophenyl)propanol (+)-tartrate and (-)-*threo*-2-tert-butylamino-1-(3-chlorophenyl)propanol (-)-

tartrate in nicotine-trained animals. Both failed to produce nicotine-like effects. Bupropion can produce a nicotine-like effect, but does not appear to do so via either of these metabolites.

**PHARMACOLOGICAL CONSEQUENCES OF ACTIVATING AND INACTIVATING MUTATIONS IN THE RAT MU OPIOID RECEPTOR.** George D. Dalton & Dana E. Selley, Department of Pharmacology & Toxicology, Va. Commonwealth University, Richmond, VA. Site-directed mutagenesis of conserved residues in the G-protein coupled rat mu opioid receptor (MOR), threonine (T279K) in intracellular loop 3 and aspartate (D114N) in transmembrane domain 2, results in a receptor that is constitutively active (T279K) or less active (D114N). Upon heterologous expression of wild-type and mutant MORs in human embryonic kidney (HEK) cells, differences in G-protein activation were seen between these receptors. In T279K-MOR cells, the relative efficacy of partial agonists in activating G-proteins was increased compared to wild-type cells in [<sup>35</sup>S]GTPγS binding assays. In fact, some compounds that are normally pure antagonists displayed partial agonist activity in the T279K mutant. The opposite effect was observed in D114N-MOR cells where the efficacy of most agonists was significantly decreased. Receptor binding studies showed that the T279K-MOR was expressed at low levels compared to WT-MOR or D114N-MOR, but exhibited more dramatic receptor up-regulation following pretreatment with the MOR antagonist naloxone. These results and results obtained through immunocytochemistry suggest the T279K-MOR is constitutively down-regulated in HEK cells.

**EVALUATION OF STATE-DEPENDENT LEARNING EFFECTS OF CLUB DRUGS.** Katriona G. Bühler, Sharon Brown, & Jenny L. Wiley, Virginia Commonwealth University, Dept. of Pharmacology & Toxicology, Richmond, VA. State-dependent learning is a phenomenon that occurs when learning has been acquired in a chemical state and can only be recalled when under the original chemical influences. In this study, we tested ketamine, a club drug that is known on the streets as Special K. Forty rats were trained to perform an adjusting ratio task in Skinner Boxes. They were divided into 4 groups and administered 5.6mg/kg of ketamine or saline daily, fifteen minutes prior to the session. Group 1 was trained under saline with results yielding no transfer of response when rats were tested under 5.6 mg/kg ketamine. Group 2 was trained under 5.6 mg/kg ketamine and generated mixed results with slightly more than 50% of the rats passing when tested under the same drug. Group 3 trained under 5.6 mg/kg ketamine and failed to show a transfer of response when tested under saline, with all animals failing the task. Group 4 trained under saline and tested under saline, resulting in all rats meeting criteria and passing on test day which indicated an expected transfer of response. The results of this study indicate rats that were switched from ketamine to saline or from saline to ketamine failed to show a transfer of response, suggesting that state-dependent learning occurred at 5.6 mg/kg of ketamine. Supported by NIDA grant DA-01442 and an undergraduate research grant from VCU.

**BINDING OF β-CARBOLINES AT SEROTONIN 5-HT<sub>2A</sub> RECEPTORS.** B. Grella<sup>1</sup>, M. Teitler<sup>2</sup> & R. A. Glennon<sup>1</sup>, <sup>1</sup>Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, VA 23298 and <sup>2</sup>Department of Pharmacology, Albany Medical College, Albany, NY 12208. A large series of β-carbolines, including several that are known to be hallucinogenic in humans, was synthesized and binding affinities for 5-HT<sub>2A</sub> receptors determined. The β-carbolines varied in their degree of saturation of the C-ring and by the position of a methoxy or bromo group in the A-ring. Their binding affinities ranged from 20 nM to greater than 10,000 nM depending upon the degree of saturation of the C-ring and the position of the substituent in the A-ring. It was determined that substituents at the 5-position (including some that are very large) significantly enhance binding affinity. In any event, and inconsistent with the 5-HT<sub>2A</sub> hypothesis of hallucinogenic activity, there does not appear to be a relationship between 5-HT<sub>2A</sub> binding affinity and hallucinogenic potency. A binding profile for a representative β-carboline (harmaline) showed that harmaline binds with very low affinity at most other receptor populations.

PYRROLIDINE-SUBSTITUTED BENZAMIDES AS POTENTIAL 5-HT<sub>3</sub>/D<sub>2</sub> ANTAGONISTS. E. De Oliveira<sup>1,2</sup>, M. Da Silveira<sup>2</sup>, M. Teitler<sup>3</sup> and R.A. Glennon<sup>1</sup>, <sup>1</sup>Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, VA 23298; <sup>2</sup>Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo SP, Brazil; <sup>3</sup>Albany Medical College, Albany, NY. Substituted benzamides have been used clinically as antiemetics (e.g. metoclopramide, mosapride) and antipsychotics (AP) (e.g. remoxipride, sulpiride). These effects are related either to 5-HT<sub>3</sub> and/or D<sub>2</sub> receptor antagonism. The structure-affinity relationships (SAFIR) of the aromatic portions of these molecules has been thoroughly investigated, but the pyrrolidine portion has been relatively unexplored. Hence we undertook an investigation of the structural requirements of the pyrrolidine moiety for 5-HT<sub>3</sub> and D<sub>2</sub> binding. Specifically, we examined several new 2- and 3-pyrrolidinyl-methylene-substituted-benzamides. The methodology chosen for synthesis of the substituted pyrrolidine ring, was the iodocyclization of  $\alpha$ - and  $\gamma$ -alkenyl- $\beta$ -enaminoesters. New derivatives are being synthesized to establish the SAFIR for this class of compounds.[Supported in part by a CAPES Fellowship, Brazil.]

CHRONIC DELTA-9 THC EXPOSURE DECREASES THE EFFICACY AND POTENCY OF THE NONSTEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDS). Rene Anikwue & Sandra Welch, Dept. of Pharmacology and Toxicology., Va. Commonwealth University., Richmond, Va 23298. Cannabinoids have been shown to increase the release of arachadonic acid metabolites and induce Cyclooxygenase-2 (COX-2) expression. We evaluated the antinociceptive effects of chronic administration of  $\Delta^9$  THC, anandamide (an endogenous cannabinoid) and methanandamide on several NSAIDS via p.o and/or i.p routes of administration using the mouse PPQ test, a test for visceral nociception. Our studies with the CB1 antagonist (SR141716A) and the CB2 antagonist (SR144528) were performed in order to better characterize PPQ interactions with cannabinoid receptors. When NSAIDs (p.o.) were administered the ED50's were as follows: aspirin 23 mg/kg, indomethacin 3 mg/kg, celecoxib 5 mg/kg, ketoralac 3 mg/kg, acetaminophen 57 mg/kg (32.3-99.8) and diclofenac 0.8 (0.1-4.9). In animals given chronic  $\Delta^9$  THC only diclofenac and acetaminophen were active. Conversely, chronic methanandamide (i.p.) did not alter the antinociceptive effects of the NSAIDs. Neither the CB1 or CB2 antagonist blocked the effects of the NSAIDs.

A QSAR STUDY OF ARYLGUANIDINES / 5-HT<sub>3</sub> INTERACTIONS. M.Khalifa<sup>1</sup>, M.Dukat<sup>1</sup>, M.Teitler<sup>2</sup> & R.A.Glennon<sup>1</sup>,<sup>1</sup>Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, VA 23298 and <sup>2</sup>Center for Neuropharmacology and Neuroscience, Albany Medical College. Arylguanidines are a novel class of compounds that interact at 5-HT<sub>3</sub> receptors. Preliminary studies in our laboratories suggested the involvement of lipophilic and electronic character in their binding interactions. A statistically valid number of 3- and 4- substituted arylguanidines were synthesized and their affinities for 5-HT<sub>3</sub> receptors were measured. A correlation was found between their pK<sub>i</sub> values, lipophilic and electronic character. It was concluded that the lipophilic nature of the 4-position substituent (n = 7, r = 0.82) is more important than its electronic character (r = 0.24), whereas for 3-substituted compounds electronic character (n = 7, r = 0.88) is more important than lipophilic character (r = 0.14). Both properties seem to play a role in 5-HT<sub>3</sub> binding.

BUPRENORPHINE SUBSTITUTION IN THE TREATMENT OF MORPHINE-DEPENDENT RAT PUPS. D. C. Stoller & F. L. Smith, Department of Pharmacology/Toxicology, Virginia Commonwealth University, Richmond, Virginia. Infants receiving ECMO or mechanical ventilation require continuous infusions of morphine and exhibit signs of withdrawal upon cessation of morphine. Currently, the morphine dose is tapered over 2- to 3- weeks to avoid withdrawal. Studies on dependent adults demonstrate that buprenorphine effectively reduces opiate withdrawal. Alzet 1003D osmotic minipumps were implanted into the subcutaneous space of post-natal day 14 rats to deliver morphine at 2 mg/kg/h. After 72-h, the effectiveness of single and repeated doses of buprenorphine was assessed in rats undergoing spontaneous withdrawal. Signs of wet-dog shakes, abdominal

stretches, and forepaw tremors were counted, and signs of splayed hind limbs, ptosis, and evoked vocalization were noted as either absent or present. Vehicle-injected rats exhibited a robust spontaneous withdrawal from morphine. A single dose of buprenorphine (1 mg/kg) administered 30-min before pump removal reduced the early signs of morphine withdrawal. Repeated doses of buprenorphine suppressed withdrawal throughout the entire 5-day period. In addition to physical dependence, 72-h of continuous morphine administration result in the development of tolerance with a complete loss in the efficacy of further morphine doses.

**GAMMA-CARBOLINE BINDING AT HUMAN 5-HT<sub>5A</sub> RECEPTORS.** N. Khorana<sup>1</sup>, M. Dukat<sup>1</sup>, M. Teitler<sup>2</sup> & R.A. Glennon<sup>1</sup>, <sup>1</sup>Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, VA 23298 and <sup>2</sup>Department of Pharmacology, Albany Medical College, Albany, NY 12208. The 5-HT<sub>5</sub> receptors subfamily was discovered in 1992 and is still not well explored. The pharmacological and biochemical mechanisms of this receptor are still unclear due to lack of selective ligands. Several reports claimed the involvement of 5-HT<sub>5</sub> receptors in migraine, psychiatric disorders, emotional control, and learning ability. There are two subtypes of 5-HT<sub>5</sub> receptors but only 5-HT<sub>5A</sub> receptors exist in humans. There is 82% amino acid identity between rat, mouse and human 5-HT<sub>5A</sub> receptors. We have identified  $\gamma$ -carboline derivatives as binding m5-HT<sub>5A</sub> receptors. These ligands were modified systematically i) to improve affinity for h5-HT<sub>5A</sub> receptors, ii) to compare affinity between human and mouse 5-HT<sub>5A</sub> receptors, and iii) to identify minimal structure requirements of the  $\gamma$ -carboline for binding at h5-HT<sub>5A</sub> receptors.

**ROLE OF ERYTHROPOIETIN-DEPENDENT AND CONSTITUTIVE PHOSPHORYLATION OF BAD ON SERINE 112 IN SURVIVAL/APOPTOSIS OF ERYTHROID CELLS.** R.M. Abutin, H. Bao, S.M. Jacobs-Helber, D.L. Barber, and S.T. Sawyer, Dept. of Pharmacology & Toxicology, VCU. Erythropoietin (EPO) is the primary hormone required for survival, proliferation, and differentiation of erythroid progenitors. A central regulator of survival/apoptosis is the BCL-2 family of proteins, which include the pro-apoptotic protein BAD. Survival factors such as EPO or mutations in cancerous cells may activate BAD protein kinases, leading to phosphorylation and sequestration of BAD with 14.3.3 to suppress BAD activity. Whereas BAD may be phosphorylated at serine residues 112, 136, and 155, we observed phosphorylation of BAD only at serine 112 in all cells tested. Tested Apoptosis-resistant cell lines maintained a constitutive phosphorylation of BAD after extended removal of EPO compared to apoptosis-sensitive cell lines. LY294002, a PI3 kinase inhibitor, blocked constitutive BAD phosphorylation with a modest decrease in EPO-dependent phosphorylation. Also LY294002 treatment of HCD57-R cells shifted the onset of apoptosis from 24 hours following EPO withdrawal to immediately. Combinations of LY294002 and other inhibitors decreased BAD phosphorylation to nearly undetectable levels but did not induce apoptosis in HCD57-R cells in the presence of EPO. In contrast to a role of constitutive BAD phosphorylation in limited survival of leukemia cells deprived of EPO, activation of BAD S112 kinase activity is not required for long term survival when EPO is present.

**SAR OF TRYPTAMINE BINDING AT 5-HT<sub>6</sub> RECEPTORS.** M.R. Pullagurta<sup>1</sup>, J.B. Rangisetty<sup>1</sup>, B.L. Roth<sup>2</sup>, M. Dukat<sup>1</sup>, & R.A. Glennon<sup>1</sup>, <sup>1</sup>Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, VA 23298 and <sup>2</sup>Department of Biochemistry, Case Western Reserve University. 5-HT<sub>6</sub> receptors belong to the family of GPCRs and are positively coupled to an adenylate cyclase second messenger system. 5-HT<sub>6</sub> receptors are believed to play a role in locomotor control, cognition and memory processes and seizure propagation. 5-HT<sub>6</sub> receptor antagonists might be useful in the treatment of several CNS disorders, memory and cognitive dysfunction, and as anticonvulsants. We earlier reported our discovery of the novel 5-HT<sub>6</sub> receptor antagonists, N<sub>1</sub>-(benzenesulfonyl)tryptamines. In the present investigation, structure activity studies were conducted to determine the influence of individual structural features of the N<sub>1</sub>-(benzenesulfonyl)tryptamines that are required for high-affinity binding. Several novel structure-types were identified that retain high affinity for 5-HT<sub>6</sub> receptors.

INVOLVEMENT OF PKC AND PKA IN MORPHINE TOLERANCE BUT NOT PHYSICAL DEPENDENCE IN MICE. R.R. Javed, F.L. Smith, M.J. Elzey, S.P. Welch, D. Selley, L. Sim-Selley & W.L. Dewey, Department of Pharmacology and Toxicology, Virginia Commonwealth University School of Medicine, Richmond, Virginia 23298. Evidence shows that the phosphatidylinositol cascade plays an important role in the expression of opioid antinociceptive tolerance. Previous studies from our laboratory demonstrated that PKC inhibitors acutely reversed a 8-fold level of morphine tolerance when injected intracerebroventricularly 30-min before the radiant heat tail-flick test. Experiments in this study attempted to determine the duration of tolerance reversal by two structurally dissimilar PKC inhibitors, Go 7874 and sangivamycin. We found that these inhibitors persistently reversed morphine tolerance for up to 24-h. However, these inhibitors failed to block naloxone-precipitated withdrawal. Other studies have shown that the PKA inhibitor, KT-5720, completely reversed 8-fold morphine tolerance. This led us to conclude that both PKC and PKA mediate morphine antinociceptive tolerance. We speculated that the individual contribution of both protein kinases to tolerance would be revealed in mice with profound morphine tolerance. We found that combined administration of both PKC and PKA inhibitors completely reversed a 50-fold morphine tolerance.

REAL-TIME-RT-PCR ANALYSIS OF RANK-L AND OPG EXPRESSION IN CULTURED FIBROBLAST AND MACROPHAGE CELLS: IMPLICATIONS FOR OSTEOLYSIS. M. Li<sup>1</sup>, J. Greenhalgh<sup>2</sup>, W. A. Jiranek<sup>3</sup> & M. J. Beckman<sup>2</sup>, Depts. of <sup>1</sup>Anatomy and <sup>2</sup>Biochemistry, <sup>3</sup>Orthopaedic Surgery and Orthopedic Research Lab, Virginia Commonwealth University, Richmond, Va 23298. Fibroblasts and macrophages have been found to be the main cell types in the periprosthetic membrane. In vitro studies have demonstrated OPG and RANK-L protein expression in the membrane using immunohistochemistry. In this study, quantitative gene expressions of OPG and RANK-L were stimulated by the IL-1 $\beta$  and TNF- $\alpha$  added into culture with U-937 (macrophage) or HS-5 cells (fibroblast).  $1 \times 10^6$  cells were incubated with IL-1 $\beta$  (50ng/ml) or TNF- $\alpha$  (50ng/ml) at various time points for 0,1,3,6,9 and 18 hrs. Real-time RT-PCR was developed to quantify the amount of RANK-L and OPG mRNA in each sample. We found RANK-L gene expression in both cell types is extremely low. OPG gene expression was higher in HS-5 than in U-937, but present in both cell types. The highest level of OPG gene expression was in HS-5 cells around 6 hr after treatment with either IL-1 $\beta$  (4-fold) or TNF- $\alpha$  (2-fold). While in U-937 cells, the OPG gene expression increased only slightly during the 1 and 3 hr for IL-1 $\beta$  and TNF- $\alpha$  treatments, respectively.

CHROMIUM SUPPLEMENTATION IN INSULIN-DEPENDENT DIABETES MELLITUS RATS. M. J. Fell & K. E. Loesser, Dept. of Biol., Mary Washington College, Fredericksburg, VA 22401. Chromium has been shown to decrease blood glucose levels of IDDM animals, potentiate the insulin activity by 10-fold or more at low levels *in vitro*, and increase the sensitivity of insulin receptors. The purpose of this experiment was to investigate the possible benefits of chromium supplementation in IDDM rats. It was hypothesized that rats placed on insulin and a chromium diet would have greater tyrosine kinase activity due to an increased amount of insulin receptors, a lower concentration of cholesterol, a higher body weight ratio (death vs. start), and a higher muscle to body weight ratio when compared to the nonchromium-supplemented IDDM rats. Chromium was given to 2 groups of IDDM rats. Each group of rats was kept for 1 month and either supplemented with  $1 \mu\text{g CrCl}_3 \cdot 6\text{H}_2\text{O}$  + insulin (n=5), insulin alone (n=4), or unsupplemented (n=6). It was determined that the IDDM rats placed on the chromium supplemented diet did not differ in any parameter, except in the muscle to body weight ratio, from other groups. The IDDM rats supplemented with insulin and chromium had a smaller gastrocnemius muscle to body weight ratio when compared to the other IDDM group. These results infer that a chromium diet does not alleviate the symptoms/conditions of IDDM.

EFFICACY OF  $\gamma$ -TOCOPHEROL VS.  $\alpha$ -TOCOPHEROL TO AMELIORATE ATHEROSCLEROTIC PLAQUE FORMATION. Sara E. Woodie & K. Loesser-Casey, Dept. of Biol., Mary Washington College, Fredericksburg, VA 22401. Atherosclerosis is one of the leading causes of death in our country. Previously, many patients were treated with the alpha form of Vitamin E to ameliorate the plaque development that lead to this disease. However, recent studies suggest this was an ineffective treatment. We hypothesized that  $\gamma$ -tocopherol would serve as a more effective preventative measure than  $\alpha$ -tocopherol because  $\gamma$ - is preferentially absorbed over  $\alpha$  and it is found in greater quantities in our natural food supply. Therefore 16 male hyperlipidemic mice (JAX strain C57BL/6J) were randomly assigned to 1 of 4 groups each fed a different diet: 1) regular chow, 2) high fat chow, 3) high fat chow supplemented with  $\alpha$ -tocopherol, and 4) high fat chow supplemented with  $\gamma$ -tocopherol. Serum lipid levels, plaque development, and weight gains were measured after the 16 weeks. Alpha -treated mice showed a small, but statistically insignificant increase in body weight in comparison with  $\gamma$ -treated mice. The serum HDL levels of  $\alpha$ -tocopherol treated mice were significantly lower (mean 28.4 mg/dL) than any other group, but no other lipid levels were statistically different. We conclude that  $\gamma$ - may be more useful than  $\alpha$ - tocopherol for increasing HDL levels.

EXPERIMENTAL MYASTHENIA GRAVIS. E. R. Printy & K E. Loesser, Dept. of Biol., Mary Washington College, Fredericksburg, VA 22401. Myasthenia gravis (MG) is a chronic autoimmune disease that affects about 14 out of 100,000 people in the U.S. In MG, auto-antibodies are produced that block, alter or destroy the acetylcholine receptors essential for muscle contraction. The specific goal for this project was to develop and characterize an animal model for the study of MG. Twelve C57BL-6 mice were immunized for the experiment, 6 with 2 injections of complete Freund's adjuvant (CFA) 25 days apart and 6 with bp129-145 of the  $\alpha 1$  subunit of the acetylcholine receptor (Bachem Pharm.) emulsified in CFA. Twenty-five days later, the gastrocnemius muscle was evaluated for muscle strength and fatigue. The isometric tension developed was measured by attaching the Achilles tendon of the mouse to a F-200 Myograph force transducer integrated to DigiMed analyzers and a computer. The muscle was externally stimulated via the sciatic nerve using 50 volt stimuli at various frequencies. It was determined that both groups of mice reacted equally well to a single stimulus but the mice with MG fatigued much faster under continuous stimulation. This experimental myasthenia gravis model is thus representative of human MG in which daily repetitive action of voluntary muscles causes weakness and fatigue quicker than normal.

### Natural History & Biodiversity

MICROHABITAT DIFFERENCES BETWEEN THE ANTS *MONOMORIUM MINIMUM* AND *M. VIRIDE* (FORMICIDAE: MYRMICINAE) IN A LONGLEAF PINE FOREST. Hannah C. Revis & Deborah A. Waller, Department of Biology, Old Dominion University, Norfolk, Va 23529. The ant *Monomorium minimum* Buckley occurs widely throughout the United States in a variety of soil types. A close congener, *Monomorium viride* Brown, is restricted to sandy soils along the East Coast. Prior to this study, *M. viride* was recorded from Florida, North Carolina and New Jersey. We collected both *M. minimum* and *M. viride* in Virginia in the Blackwater Ecological Preserve in Isle of Wight County by using pitfall traps and ant baits along two 20 m transects set 50 m apart in two sites. *Monomorium viride* was collected in Site A, an open dirt road, and *M. minimum* was collected in Site B, a woodland trail. Habitat data, including soil and vegetation characteristics and canopy cover, were examined along the transects. Five soil classes were measured; the two smallest classes were significantly different between the sites. Vegetation species composition also differed significantly between the sites, but the species diversity indices were not significantly different. Percent canopy cover was significantly greater over Site B where *M. minimum* was found. Future research will focus on whether these habitat differences are of biological importance to the ants.

A DEMOGRAPHIC ANALYSIS OF THE SNAIL *LEPTOXIS CARINATA* IN AN APPOMATTOX RIVER TRIBUTARY: MOVEMENT PATTERNS VARY AS A FUNCTION OF HABITAT TYPE. Lori M. Brantley, Tim Stewart & Tiffany R. Edwards, Department of Natural Sciences, Longwood College, Farmville, VA 23905. An ecological study of a common river snail, *Leptoxis carinata*, was conducted from August 2001 to November 2001. One hundred snails were collected from two different sites (slow run, riffle) within Fishpond Creek, a tributary of the Appomattox River, Virginia. Snails were marked with bee tags and returned to the site of capture. Over a 28-day period, marked snails were tracked to evaluate substratum preference, and movement direction and distance as a function of habitat type. From a previous study we determined that these snails prefer large substrata, such as boulders and cobble, that have large surface areas for algal growth (their main food source) and that also provide refuges in the event of heavy flooding and strong currents. Consistent with hypotheses, snails inhabiting the slow run site, which was dominated by gravel, sand and silt substrata, demonstrated significant upstream movement as they likely searched for higher quality habitat. In contrast, negligible movements occurred among snails at the riffle site that already occupied good habitat.

COLONY DISTRIBUTION OF THE FUNGUS-GROWING ANT *TRACHYMYRMEX SEPTENTRIONALIS* RELATED TO LIGHT AVAILABILITY IN A LONGLEAF PINE HABITAT. Jonathan P. Howell & Deborah A. Waller, Biology Department, Old Dominion University, Norfolk, VA 23529. *Trachymyrmex septentrionalis* represents the only species of fungus-growing ant in the southeastern United States. These ants are common in Florida, but increasingly scarce as they near the northern extent of their range in New Jersey. This study was performed in the Blackwater Ecological Preserve where approximately 160 active nests have been monitored since April 27, 2001. Colonies were seasonally active for 13 weeks, from April 27 to October 12. Data from three geographically distinct populations were analyzed for this study. Population densities ranged from 0.102 to 0.134 nests/m<sup>2</sup> and were significantly affected by ground cover. Further analysis of ground cover found a significant relationship between cover and soil and air temperature and humidity. Previous research has demonstrated that soil and air temperature, humidity, and light intensity have a significant impact on the surface activity of the ants.

COMPARISON OF DEN SITE CHARACTERISTICS FOR NORTHERN FLYING SQUIRRELS IN TWO DIFFERENT AGE STANDS IN MT. ROGERS NRA, VIRGINIA. Mundy Hackett & John F. Pagels, Department of Biology, Virginia Commonwealth University, Richmond, VA 23284. In the Southern Appalachians, the endangered northern flying squirrel (*Glaucomys sabrinus coloratus*) occupies remnant patches of red spruce (*Picea rubens* Sarg.) interspersed with northern hardwoods. Populations of *G. s. coloratus* are limited in Virginia to the Mt. Rogers National Recreational Area (NRA). To date, few studies have provided quantitative information on den site characteristics of *G. s. coloratus* in the southern Appalachians. During 2000-2002, we used telemetry to locate 25 den sites for 12 squirrels. Den sites included cavities in live and dead residual growth trees (dbh > 35 cm dbh); cavities, and drays in mature growth trees (10-35 cm dbh); and dens in subterranean root masses of fallen trees or in rock falls. All dens located in dead trees at our two study sites, Whitetop Mountain and Cabin Creek, were yellow birch (*Betula alleghaniensis* Britton.). Ninety-five percent of dens located in live trees at Cabin Creek were in yellow birch. Forty-five percent of dens located in live trees at Whitetop Mountain were in yellow buckeye (*Aesculus octandra*). Subterranean nests were utilized 28% and 29% of the time respectively at Cabin Creek (n=5) and Whitetop Mountain (n=2).

TRENDS THROUGH TIME: 57 YEARS OF SPRING ARRIVAL DATES FOR NEOTROPICAL MIGRANTS IN CENTRAL VIRGINIA. P. A. Bedell, 10120 Silverleaf Terrace, Richmond, VA 23236. There is recent evidence that the breeding phenology of some species of birds may be responding to climate change. This response may be reflected in the spring arrival dates of neotropical migrants. I assembled arrival dates for 43 locally common species of neotropical migrants



in central Virginia spanning the years 1945 - 2001. Regression analysis showed 22 species arriving significantly earlier over the 57 - year period. Twenty species showed no significant change, and only one, the American Redstart (*Setophaga ruticilla*), trended later ( $n = 52$ ,  $t = 2.05$ ,  $P = 0.046$ , slope = 0.111 days per yr). The Eastern Pewee (*Cantopus virens*), though not significant at  $P = 0.05$ , is also trending later ( $n = 54$ ,  $t = 1.88$ ,  $P = 0.064$ , slope = 0.064 days per yr). Detectability of migrants by field observers may be a reflection of changing population size. But there was no association between arrival trends and North American Breeding Bird Survey population trends ( $df = 2$ ,  $\chi^2 = 0.605$ ,  $P = 0.717$ ).

ASSESSMENT OF BIOLOGICAL INTEGRITY IN AN AGRICULTURALLY IMPACTED VIRGINIA MOUNTAIN STREAM. J. H. Roberts<sup>1</sup>, T. J. Newcomb<sup>1</sup>, & M. J. Pinder<sup>2</sup>, <sup>1</sup>Department of Fisheries and Wildlife Sciences, Virginia Tech, Blacksburg, VA 24061 and <sup>2</sup>Virginia Department of Game and Inland Fisheries, Blacksburg, VA 24060. The Glen Alton Tract (GAT), a recent U.S. Forest Service acquisition in Giles County, Virginia, contains approximately three km of Big Stony Creek. One section of the GAT has a history of agricultural impacts, stemming from unrestricted cattle access to the stream. We sought to determine the effects of these impacts on the fish assemblage and the biological integrity of Big Stony Creek. Six fish sampling sites were distributed equally among an upstream reference reach, a central impact reach, and a downstream recovery reach. We applied the Bray-Curtis similarity coefficient to each site and determined that fish assemblage variability was higher among than within reaches. We also applied an Index of Biotic Integrity (IBI), modified for expected regional conditions, to each site. Integrity scores were generally low and decreased between upstream and downstream sites. Our study indicated agricultural impacts to the fish fauna of Big Stony Creek; however, difficulties associated with assessing biological integrity in naturally depauperate headwater streams hampered our ability to apply the IBI within the GAT.

ANT CATCH RELATED TO PITFALL TRAP TYPE IN A LONGLEAF PINE HABITAT. Deborah A. Waller, Department of Biology, Old Dominion University, Norfolk, Va 23529. Ants perform important functions in ecosystems such as predation, seed dispersal, pollination, soil aeration and nutrient translocation. Pitfall traps are one of the most effective methods of sampling ants, but trap design has received little attention. In the present study I compared ant catch in two trap types: empty 250 ml cups and 16 x 50 mm testtubes partially filled with soapy water. The study was conducted at the Blackwater Ecological Preserve in Isle of Wight County, Virginia, in August 2001. Two perpendicular 100 m transects were placed in each of two sites. Every 10 m along the transects, one testtube and one cup trap were sunk level to the ground. Traps were collected after one week, replaced, and collected again the following week. Ants were identified to species in the laboratory. The testtube and cup traps collected 26 and 11 ant species, respectively. No ant species were collected in the cups that were not present in the testtubes. Significantly more ant species were collected in testtubes than in cups at each trap location. In addition to collecting more ants, testtube traps are more compact, portable and durable than cup traps, and they pose less danger to vertebrates which can inadvertently fall into the cups.

LIFE HISTORY ASPECTS OF THE CREEPER, *STROPHITUS UNDULATUS*, AND GREEN FLOATER, *LASMIGONA SUBVIRIDIS* (BIVALVIA: UNIONIDAE). R. Mair, J. W. Jones, and R. J. Neves, Department of Fisheries and Wildlife Sciences, Virginia Tech, Blacksburg, Virginia 24061. Freshwater mussels have a unique life cycle that requires the use of a host fish to transform their larvae (glochidia) into juveniles. Both of these mussel species have large hooked, triangular glochidia that are capable of both directly transforming into juveniles inside the gills of the female mussel, and by using a host fish. *L. subviridis* was once common in Virginia, but now it is becoming increasingly rare in the state. However, this mussel species still can be found in the Potomac, Rappahannock, York, James, Chowan, New, Dan and Roanoke River systems. It prefers areas in rivers with slow to moderate flow in a substratum of sand and fine sediments. This mussel species also is a hermaphrodite. Fecundity of four females ranged from 6,000-12,000 glochidia per mussel. *S.*



*undulatus* is widely distributed in streams throughout Virginia. It inhabits areas in streams with moderate to slow flow, also preferring a substratum of sand and fine sediments. This mussel is capable of using many species of fish as host to its glochidia. Fecundity of three *S. undulatus* females ranged from 12,000-42,000 glochidia per mussel. Glochidia were contained in conglomerates which ranged in length from 2.95-3.28 mm. Glochidia length ranged from 0.38-0.43 mm. Glochidia were observed sliding-out of conglomerates 2-10 minutes after they were removed from the gills of the female mussel. The movement of glochidia out of the conglomerates seems to be related to kinetic energy produced by the expansion of conglomerates as they absorb water.

USING ZEBRAFISH SURVIVORSHIP TO MONITOR ORGANIC POLLUTANTS IN SEDIMENTS OF VIRGINIA WATERS. Robert M. Northington & Timothy W. Stewart, Department of Natural Sciences, Longwood College, Farmville, VA 23909. Human activities have led to increased industrial pollution of the environment. Many of these chemicals, when dumped into aquatic ecosystems, bind to sediment particles at the bottom of the water systems. When benthic-feeding organisms feed from this contaminated sediment, abnormalities may result in subsequent generations. These abnormalities may eventually lead to the organism's death. The effects of chemical pollution can be seen in the laboratory using model organisms, such as zebrafish (*Danio rerio*). Zebrafish embryo survivorship in pore water extracted from aquatic environments in Lake Michigan sites was analyzed. Statistical analyses indicated nearly significant ( $p = 0.055$ ) mean survivorship between water treatments. Further studies of embryo survivorship in Virginia sediment samples will be carried out in the future. These studies will help to assess the level of sediment contamination using samples from ecosystems with differing levels of industrialization. Zebrafish survivorship will be used as an indicator of the level of toxicity of different sediments.

EFFECT OF PRESCRIBED BURNS ON INSECT DIVERSITY IN A LONGLEAF PINE HABITAT. Charles F. Abadam & Deborah A. Waller, Department of Biology, Old Dominion University, Norfolk, Va 23529. The Blackwater Ecological Preserve, the northernmost stand of reproducing longleaf pine, is managed with prescribed burns. The objective of the present study was to examine the impact of the burns on the insect fauna. Four malaise traps were installed at the preserve, two in areas that were burned in 2001 and two in control areas that had never been burned. Traps were collected every week beginning February 15, 2002. The insect catch was preserved in alcohol and later sorted to order. Soil and air temperature were measured weekly at each trap site. Results were tabulated through April 19, 2002. Total numbers of insects did not differ for burned and control sites, but different orders varied significantly in number, with Diptera the most numerous. There was a significant increase in numbers of insects in all orders over time. Numbers of Hymenoptera were significantly greater in the control sites than in the burned areas. However, there was no difference between burn and control sites for numbers of Coleoptera, Diptera, Homoptera or Lepidoptera. Both soil and air temperature increased significantly over time. Air temperature was significantly greater than soil temperature, but there was no difference in either air or soil temperature between burned and control sites.

SUBTERRANEAN TERMITE (ISOPTERA: RHINOTERMITIDAE) RESPONSE TO ESSENTIAL OILS. Laura K. Baron & Deborah A. Waller, Department of Biology, Old Dominion University, Norfolk, Va 23529. Subterranean termites are important structural pests in urban ecosystems. New baiting technologies show promise for termite control, but drawing termites to bait stations is often problematic. Chemical attractants might increase bait effectiveness. We examined the response of the Eastern Subterranean Termite, *Reticulitermes flavipes* Kollar, to different essential oils (four pure oils and one blend). Experimental units consisted of a central 50 cc cup connected by straws to four similar cups (two treatment and two control cups). All cups were filled with 40 cc sterilized sand and 10 ml deionized water. Nine termite workers and one soldier were added to the central cup. Each treatment and control cup received a 6 mm diam red paper circle. A drop of essential oil was added to the treatment paper, and the control paper was untreated. Two

experimental units were set up for each of six *R. flavipes* colonies for each oil. After one week, workers and soldiers in treatment units were counted and categorized as either live white (unfed), live red (fed on red paper), dead white (unfed) or dead red (fed on red paper before dying). Different oils had significantly different proportions of these termite categories.

**ANURAN OVIPOSITION SITE SELECTION: HOW BEHAVIOR INFLUENCES COMMUNITY STRUCTURE.** J. F. Rieger, C. A. Binckley & W. J. Resetarits Jr., Department of Biology, Old Dominion University, Norfolk, VA, 23529. While patterns of larval amphibian distributions and abundances have been ascribed to direct predation, adult oviposition site selection is an alternative mechanism which can produce identical larval distributional patterns. Such behavioral habitat selection has been documented in several amphibian species whose larva lack anti-predator defenses. However, the sensitivity of adult amphibians to detect predator cues and avoid these habitats has not been determined. Furthermore, if larval survivorship is greatly reduced in sites containing fish, there should be strong selection to detect and avoid habitats with low fish densities. We conducted two experiments, which (1) evaluated the ability of ovipositing *Hyla femoralis* to detect varying densities of a predatory fish (*Umbra pygmaea*), and (2) quantified larval performance under varying densities of these predators. Experiments utilized wading pools in a complete randomized block design with treatments levels of 0, 1, 2, 3, 4, 5, and 6 individual fish per pool. Data from the two experiments suggest that *H. femoralis* can detect low densities of fish and oviposit in habitats that result in higher offspring survivorship.

**HABITAT USE AND EXPLOITATION OF THE STRIPED BASS AND HYBRID STRIPED BASS IN CLAYTOR LAKE, VIRGINIA; PRELIMINARY FINDINGS.** John M. Kilpatrick, Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University, Blacksburg, Va 24061. We investigated the habitat use, movements, and exploitation of striped bass and hybrid striped bass in Claytor Lake Virginia. The following is preliminary data collected during 2001. Striped bass (SB) ( $n = 9$ ) and hybrid striped bass ( $n = 11$ ) (HSB) were fitted with temperature sensitive radio tags and located bi-weekly. In addition, SB ( $n = 19$ ) and HSB ( $n = 136$ ) were fitted with internal anchor reward tags to determine angler exploitation. There were 10 reported recaptured HSB and 3 recaptured SB, for respective 7.4% and 15.8% exploitation rates. We found an insignificant difference ( $p = 0.112$ ) in temperature use and a significant difference ( $p = 0.004$ ) in dissolved oxygen use over the time periods sampled for SB and HSB. There is significant evidence ( $p = 0.001$ ) that a difference exists for the interaction by species and month for temperature and dissolved oxygen use. Home range and movement calculations will be determined following collection of a full year of data. These results are meant as preliminary findings only and are based on only a partial year of data and small sample size. Therefore, extreme caution should be used when interpreting statistical results.

**AN UNUSUAL COAT COLOR PATTERN IN A NORTHERN SHORT-TAILED SHREW, *Blarina brevicauda*.** George B. Bumann and Patrick F. Scanlon. Department of Fisheries and Wildlife Sciences, Virginia Tech, Blacksburg, VA 24061-0321. On 12 November 1999, a small mammal specimen was brought to our laboratory and identification of the specimen was requested. The mammal was a victim of the presenter's cat and had been caught a few days earlier within the town limits of Blacksburg, VA. The specimen was identified as a northern short-tailed shrew, *Blarina brevicauda*, and was remarkable in that a considerable portion of the fur covering the abdomen was white in color. Some white fur extended to the flanks of the shrew and the dorsal surfaces of both hind feet had some white fur. The specimen here differed from that in a recent report on a white-belted coloration pattern in a *B. brevicauda* specimen from Henry County, VA, (Moncrief and Anderson, Am. Midl. Nat. 137: 397-400) in that the white coloration is mostly ventral with only a limited amount of white coloration extending laterally on both flanks while their specimen had dorsal white coloration. Walter Niehaus kindly provided the specimen.

## Psychology

AT-RISK STUDENT ALCOHOL USE: ASSESSING THE EFFECTS OF FRONT-LOADING AT FRATERNITY PARTIES. I.J. Ehrhart, M. L. Stuart, S. R. Rayne, & S. M. Krepinevich. Dept. of Psc., VA Tech. Alcohol contributes to the leading causes of accidental death in the United States, such as motor vehicle crashes and falls. This study examined the effect of front-loading (consuming alcohol before going to a party) on blood alcohol concentration (BAC). Data were collected at seven different fraternity parties over three semesters (N = 708). The mean BAC for all participants was 0.083. The mean number of drinks front-loaded was 3.16. Results showed that Front-Loaders were more intoxicated than Non-Front-Loaders ( $p < .001$ ). Front-Loaders had a mean BAC of 0.092 while Non-Front-Loaders had a mean BAC of 0.067. It was also found that males Front-Loaded more than Females. Males had an average of 3.82 drinks while females had an average of 1.77 drinks before attending a party. An analysis of variance showed a Gender by Greek Status interaction ( $p < .05$ ). Greek men Front-Loaded less than non-Greek men. Conversely, Greek women Front-Loaded more than non-Greek women. Implications suggest that Front-Loading contributes significantly to overall BAC for the evening.

ALCOHOL FRONT-LOADING: AN ASSESSMENT OF NEGATIVE OUTCOMES AND DESIGNATED DRIVER USE. M.L. Stuart, I.J. Ehrhart, B.A. Kain, & T.E. Smith. Dept. of Psych, VA Tech. Students were randomly approached in a bar setting and asked questions regarding front-loading (drinking alcohol in preparation of going to a bar), their experience with negative outcomes, and designated driver use. They were then administered a breathalyzer test to assess their actual blood alcohol concentration (BAC). A total of 294 participants were interviewed. Of these, 43 said they had only front-loaded that evening, whereas 159 said they had front-loaded and consumed alcohol while in the bar, and 68 had only consumed alcohol while in the bar. The mean BAC for all participants was .081. The mean BAC level of those who only front-loaded was .058, whereas the mean BAC of those who front-loaded and consumed alcohol in the bar setting was .103, and those who only consumed alcohol in the bar setting had a mean BAC of .074. The mean number of negative outcomes of those who only front-loaded was 2.95, whereas the mean number of negative outcomes of those who front-loaded and consumed alcohol in the bar setting was 4.39, and those who only consumed alcohol in the bar setting had a mean number of negative outcomes of 2.71. Implications of these findings for preventing driving under the influence of alcohol (DUI) are discussed.

TEST-RETEST RELIABILITY OF THE DULA DRIVING INDEX. D.J. Ramsey, C.S. Dula, & S.M. Krepinevich. Dept. of Psc. VA Tech. Dangerous driving is a serious problem that plagues our country and is becoming more prevalent with the increase of the use of motor vehicles. We are attempting to provide a measure that would assess personality traits as being a factor in dangerous driving. In this study we will be determining the test-retest reliability of a dangerous driving measure to demonstrate that dangerous driving is a personality trait and not a transient state of mind. The study will consist of undergraduate students at Virginia Polytechnic and State University enrolled in a psychology course. The students will participate in two separate testing sessions, spaced four weeks apart. To assure confidentiality students will be tracked from time one to time two by using the last four digits of their social security number and the first two letters of their mother's name. Upon the completion of the first testing session students will receive one point and after completion of the second testing session they will receive two additional points and one dollar. The answers from time one and time two will be scored and compared to establish the test-retest reliability.

PERSONALITY CHARACTERISTICS OF AGGRESSIVE DRIVERS. Michelle L. Rose<sup>1</sup>, Bryan E. Porter<sup>1</sup>, and Thomas D. Berry<sup>2</sup>, <sup>1</sup>Dept of Psych, Old Dominion University, Norfolk, VA, 23529-0267 and <sup>2</sup>Dept of Psych, Christopher Newport University, Newport News, VA, 23606. This study explored the relationship between personality characteristics and aggressive driving. Aggressive driving, "the operation of a motor vehicle in a manner which endangers or is likely to endanger people

or property” is perceived to be one of many factors that is attributed the growing number of crashes on the roadways (National Highway Traffic Safety Administration, 1998, p. 1). Researchers have shown that there may be a number of personality characteristics associated with aggressive driving. This study attempted to add to this growing literature. It was hypothesized that there would be a positive relationship between aggressive driving and an individual’s personality characteristics, such as stress, anger, aggression, and sensation seeking. A self-report survey was distributed to 127 college students at a large southeastern university during the spring semester of 2002. A logistic regression showed that high levels of anger was the only characteristic predicting whether individuals would pass on the shoulder when traffic was congested  $\chi^2(1) = 4.53$ ,  $p < 0.05$ ,  $R^2 = 0.04$  and that individual’s believed that tailgating is an effective way of moving traffic to the right lane  $\chi^2 = 6.18$ ,  $p < 0.05$ ,  $R^2 = 0.05$ .

A COMPARISON OF TAILGATING PREVALENCE ON LOCAL TWO-LANE AND FOUR-LANE ROADS IN SOUTHEASTERN VIRGINIA. Jennifer M. Piver, Kristie L. Hebert & Bryan E. Porter, Department of Psychology, Old Dominion University, Norfolk, VA 23529-0267. This study is part of an ongoing effort to increase driver awareness of risky behaviors such as tailgating. Between February and April of 2002, several two-lane and four-lane roadways were observed in Southeastern Virginia. Drivers’ mean headways (following distances behind other vehicles) were observed. Three classifications of drivers were created: followers (headways  $\leq 4$  seconds); tailgaters (headways  $< 2$  seconds); and dangerous tailgaters (headways  $< 1$  second). Two-lane roads had significantly more followers than four-lane roads, 73.6% versus 65.0%, respectively. For close followers, there were more tailgaters and dangerous tailgaters on four-lane roads than two-lane roads. It is important to note that even though there were more followers on two-lane roads, this did not translate into more tailgating. The headways for followers, tailgaters, and dangerous tailgaters were all less on four-lane roads than two-lane roads. In future research, we hope to discover why there are more followers on two-lane roads but more tailgating and dangerous tailgating on four-lane roads.

WHO’S ON MY BUMPER? : DEMOGRAPHIC PREDICTORS OF INDIVIDUALS WHO FOLLOW TOO CLOSELY. Courtney P. Schubert, Kristie L. Hebert, Sarah A. Matthews & Bryan E. Porter, Department of Psychology, Old Dominion University, Norfolk, VA, 23529-0267. The current study examined the relationship between the risky driving behavior of following too closely (tailgating) and demographics (safety belt use, gender, and vehicle type). Between May and September of 2000, 3,160 vehicles were observed in Southeastern Virginia along a four-lane divided highway and an interstate site. Demographics, as well as following distance between vehicles (headway), were observed and recorded. Vehicles with headways  $\leq$  four seconds - "followers" who had the opportunity to tailgate - were used for analyses ( $n = 2,448$ ). A multiple regression analyzing mean headways found only vehicle type contributed significant variance. SUVs and light trucks had smaller headways than passenger cars. Vehicles with headways less than two seconds were then classified as tailgaters. A logistic regression analysis indicated that drivers on the interstate were 1.23 times more likely to be tailgaters than drivers on four-lane roads, and drivers in SUV’s and small trucks were 1.22 times more likely to be tailgaters than drivers in passenger cars. Gender and belt use did not predict tailgating classification.

DEMOGRAPHIC PREDICTORS OF TAILGATING RELATED CRASHES. Sommer N. Thompson, Michelle L. Rose, Bryan E. Porter, Department of Psychology, Old Dominion University, Norfolk, VA, 23529-0267. This study attempted to create a demographic profile of tailgaters whose behavior led to crashes. As part of a larger study in 2001, police crash reports from tailgating-related incidents were collected from southeastern Virginia cities over a three-week period. Five hundred and sixty such reports were collected. Overall, there were 43.8% female and 56.2% male drivers who caused these crashes. Their mean age was 32.01 years. Results indicated women and young drivers were more likely to be cited for tailgating crashes. Other contextual variables indicated that tailgating crashes occurred more on Wednesdays and during the afternoons. In addition, these tailgating

crashes were compared with Virginia crashes at large to better understand if tailgating involved different crash predictors.

EFFECTS OF EXPOSURE TO *PFIESTERIA SHUMWAYAE* TOXIN AND SCOPOLAMINE ON RAT PERFORMANCE IN THE DELAYED RADIAL-ARM MAZE. Sarah A. Schultz<sup>1</sup>, Perry M. Duncan<sup>1</sup>, Brian Dyer<sup>2</sup>, and Howard Marshall<sup>2</sup>, <sup>1</sup>Department of Psychology, Old Dominion University, Norfolk, VA and <sup>2</sup>Department of Biological Sciences, ODU. *Pfiesteria shumwayae* is a recently identified toxic dinoflagellate. The ichthyotoxic properties of this organism have been established but the neurotoxic effects of exposure to this organism are unknown. The purpose of this experiment was to investigate the toxic effects on behavior and cognition in rats exposed to *pfiesteria* by toxic filtrate injection. The researchers utilized a delayed radial-arm maze procedure that allows testing for both reference memory errors (RME) and working memory errors (WME). Pf-exposed rats make significantly more total errors (RME + WME) than control rats exposed to a control filtrate injection. Scopolamine is a cholinergic (muscarinic) antagonist drug that has been previously shown to impair memory in humans and animals. Pf-exposed rats perform more poorly than controls in the delayed RAM procedure after Scopolamine injection but this finding only indicated a non-significant trend. It seems that Pf-exposed rats are more sensitive to Scopolamine's effect but more research is needed before this can be conclusively stated.

AN INVESTIGATION OF CONTROL CONDITIONS IN ASCH-TYPE EXPERIMENTS: III. Neysa L. Isler, Natalie A. Clouser & James P. O'Brien, Tidewater Community College, Virginia Beach VA 23456. Results are reported for the third year of a study to validate Asch's (1951, 1956) independence-conformity stimuli for clarity in a 2x2x2x2 protocol (S's sex and 4-yr. undergraduates vs. community college students; authoritative vs. peer experimenter and E's sex). With the addition of 105 Ss this year [earlier results reported in *Va. J. Sci.*, 51 (2), p. 132 & 52 (2), pp. 127-128], data has been acquired for 322 Ss and 3 of 16 cells have been completed with  $n \geq 37$  (Asch's control condition N). Measures (mean error and % Ss error-free) are more extreme than Asch's, indicating that his stimuli do not constitute "an utterly clear perceptual fact" for all people in various situations. Yet dozens of subsequent investigators, in replicating only Asch's experimental conditions without contemporaneous and comparable controls, have assumed stimulus clarity. In fact, since we have found small error measures, similar to Asch's, only with white male college undergraduates (like his controls); conclusions from numerous experimental condition replications – such as, women conform more than men – may be erroneous. After all, as Asch demonstrated, "with diminishing clarity of the stimulus-condition the majority effect increases" in the group-pressure condition.

AN EXPLORATORY ANALYSIS OF ABERRANT COMPANION ANIMAL BEHAVIORS ASSOCIATED WITH RELINQUISHMENT. A. L. Cincotta, H.B. Sewell, A. K. Fournier, & E. S. Geller. Dept. of Psc., VA Tech. Millions of companion animals are relinquished to shelters and euthanized each year because of aberrant pet behaviors and pet owners' personal reasons. The present study explored behavioral determinants of pet relinquishment at two local shelters. Data collection incorporated archival data, evaluating demographic information of each animal and the adoption and relinquishment records of animals in two animal shelters during 2001. 1,379 records of individual animals were evaluated, and animals were classified by reasons admitted to shelter, reasons for relinquishment, age, and outcomes of relinquished animals. Results showed 34.3% of animals in shelters were relinquished by their owners. It was predicted that the most frequent reason reported for animal relinquishment would be aberrant animal behaviors. The majority of the owners reported personal reasons to be the reason for relinquishment; the second most common reason was aberrant pet behaviors. Implications for future educational and behavioral interventions are discussed.

A COMPARISON OF RECIPROCITY VERSUS THE THREE-TERM CONTINGENCY IN SOLICITATION APPROACHES. S. L. Rosti, J. S. Hickman & S. E. Geller, Dept. of Psych., Va. Polytechnic Inst. & State Univ., Blacksburg, Va 24061. The current study assessed the percentage of pedestrians solicited in three different conditions, Reciprocity (R), 3-Term Contingency (3T), and Control (C) solicitation approaches. A total of 453 pedestrians participated in the study located on the Virginia Tech campus. During spring 2002, pedestrians were approached in the Derring Hall first floor lobby and outside the Newman library using one of the three solicitation approaches (R, 3T, or C). In the R approach, pedestrians were given a free gift, (i.e., an American flag pin). Upon the pedestrian's acceptance of the pin, they were asked to complete a brief survey. In the 3T approach, pedestrians were asked if they wanted to complete a brief survey and upon completion would receive a small gift (i.e., an American flag pin). The C approach was similar to the 3T approach, except no small reward was given upon completion of the survey. The results show that pedestrians solicited with the R approach agreed to complete the survey on 39.6% of the trials, 25.5% in the 3T approach, and on 29.2% of the trials in the C approach. Results showed that pedestrians who were solicited with the R approach completed the survey significantly more often than pedestrians solicited in the 3T condition.

SOCIAL INFLUENCE: CONSIDERATION OF SPECIAL INTEREST GROUPS. S.M. Krepinevich, C.S. Dula, D.J. Ramsey, & J.G. Franks. Dept. of Psych. VA Tech. This study incorporated the six social influence principles, as they are described by Cialdini (2001), into a survey that was then administered to 853 Introduction to Psychology students, 35 cadets from the universities Corps of Cadets, and 41 members of a service fraternity. The results found that the survey used, the Social Influence Survey, had external validity. The cadets scored significantly higher in authority, as was hypothesized, and the service fraternity though not significantly different from the Introduction to Psychology students, was significantly higher in reciprocity than the cadets. Both the cadets and the service fraternity were overall more influenceable than the Introduction to Psychology students, which was also hypothesized. All of this suggests not only that the survey was measuring what it was believed to be measuring, but also that the individual social influence principles are trait like where members of these groups as a whole appear to have more similar than dissimilar profiles.

SOCIAL INFLUENCE SURVEY: EMERGING PATTERNS IN STUDENT POPULATIONS AND THE DEVELOPMENT OF A NOVELTY SCALE. P. K. Lehman, C. S. Dula, E. S. Geller, D. Grandin. Dept. of Psych., VA Tech. Social psychologists have documented several social influence principles that have a profound impact on human behavior and are frequently used as compliance tools by sales and marketing professionals. A scale designed to measure individual differences in propensity to be influenced by Novelty was developed and added to an existing Social Influence Survey (SIS) designed to measure individual differences in susceptibility to the influence principles of Consistency, Reciprocity, Ingratiation, Conformity, Authority and Scarcity. To construct the Novelty scale, seventeen questions were created to measure preference for Novelty across a variety of situations and administered in Likert scale format to 103 undergraduate students. Factor analysis was used to select 7 items for the final scale. The complete SIS was administered to 614 undergraduate students in an introductory psychology course. Mean scores were highest for the Consistency (40.9) and Reciprocity (40.4) scales, followed by Novelty (33.0), Authority (31.6), Scarcity (31.3), Ingratiation (30.5) and Conformity (20.6). Mean scores for females were significantly higher than those for males on all scales except Conformity and Ingratiation ( $p < .01$ ).

SAFETY SELF-MANAGEMENT IN MINING OPERATIONS. J. S. Hickman, E. S. Geller, A. L. Cincotta, S. L. Rosti, S. L. & D. Grandin, Department of Psychology, Virginia Polytechnic Institute and State University, 5100 Derring Hall, Blacksburg, VA 24061. This quasi-experimental field study examined the efficacy of a safety self-management intervention to increase safety-related work practices in mining operations. A total of 15 male miners participated in the study while engaging

in their normal work practices at the Virginia Tech Quarry, located in Blacksburg, Virginia. The study had two groups, *Feedforward* ( $n=8$ )--participants self-recorded their intentions to engage in specific percentages of safety-related work behaviors *before* starting their shift for the day, and *Feedback* ( $n=7$ )--participants self-recorded their percentages of safety-related work behaviors *after* their shift for the day. Trained research assistants made a total of 10,905 obtrusive behavioral observations on three target behaviors (ear plugs, dust mask, and safety glasses) and five non-target behaviors (gloves, hard hat, boots, knee position during lifts, body position during lifts) across phases. Results showed the safety self-management intervention significantly increased safety performance across both target and non-target behaviors during the Intervention phase.

AGE DIFFERENCES IN APPROPRIATE NAVIGATIONAL COMMAND DISTANCE: A DRIVING SIMULATION STUDY. Rebecca D. Ferris<sup>1,2</sup>, Carryl Baldwin<sup>1</sup> & Barbara Freund<sup>2</sup>. <sup>1</sup>Dept. of Psychology, Old Dominion University, Norfolk, VA 23507, <sup>2</sup>The Glennan Center, Eastern Virginia Medical School, Norfolk, VA 23507. Using auditory commands as an aid for older adults using in-vehicle navigation systems may be a safer means than using visual displays. Command distance was examined in an attempt to find an ideal distance from turn command to intersection. Participants were 16 older (age 65-80) and 16 younger (age 20-35) adults in self-reported good health with at least 3 years of driving experience, screened for dementia and hearing loss. Each participant drove 3 driving simulator scenarios--either short, medium or long command distances and completed a subjective questionnaire (NASA-TLX) as a measure of self-reported mental workload. A significant number of the older participants failed to complete turns during the short scenario, possibly due to feelings that they would not complete the turn safely or the older adults may not have had sufficient time to cognitively process the turn command before arriving at the intersection. The NASA-TLX was also significant. All participants rated the short distance the most difficult, medium less difficult and long the least difficult, with the older group ratings significantly higher than the young group overall.

ALARM RELATED INCIDENTS: A REVIEW OF THE U. S. ARMY AVIATION SAFETY DATABASE. Colleen M. Moore & James P. Bliss, Dept. of Psychology, Old Dominion University, Norfolk, VA 23529. In the complex environment of today's aviation cockpits, it is important to have reliable alarm systems. In the situation of an alarm, the pilot must be able to make a quick and accurate assessment of the alarm as well as continue with his or her primary tasks. Studies have shown "cry-wolf" effects and delays in response to alarms and mistrust in alarm systems in a laboratory setting. However, there is no applied research due to the difficulty of performing studies onboard aircraft. Therefore, accurate alarm rates are difficult to determine. The following study reviewed the U.S. Army aviation safety database for incidents and accidents involving warning signals. Researchers focused on false and missed alarms. Of the 479 cases reviewed 90% were false alarms and 4% were missed alarms. These results suggest sensitive warning systems in Army aircraft.

LEADERSHIP STYLE AND PERFORMANCE IN TELEWORK: A SECONDARY DATA ANALYSIS. Rebecca D. Say, & Donald D. Davis, Dept. of Psychology, Old Dominion University, Norfolk, Virginia 23529. Telework encompasses those options available to persons who work outside of the traditional office. Teleworkers and those persons who lead teleworkers must have specific characteristics to ensure success. The present study examined the impact of two types of leadership, transactional and transformational leadership, on teleworker task and contextual performance using data collected via an on-line questionnaire. Although relationships between leadership and employee performance have been previously identified for on-site workers, it is important to determine their application to teleworkers. The sample includes 36 teleworkers from two companies. Results of two sequential multiple regressions indicate that after controlling for average number of days teleworked, transactional leadership significantly predicted both task and contextual performance of teleworkers. Results also indicate that transformational leadership did not significantly predict either performance type. These findings suggest that transactional leadership



methods may be the most effective way to lead teleworkers. Further research should be conducted to determine the precise relationships between leadership and performance for teleworkers.

**HOW ECONOMIC FACTORS ARE RELATED TO ARMY RETENTION RATES: A TIME SERIES ANALYSIS.** James R. Camic & Robert M. McIntyre, Psychology Department, Old Dominion University, Norfolk, VA, 23529. The relationship between economic factors and Army retention rates was examined using a time series analysis. The analysis was conducted using archival data drawn from fiscal year 1979 through 1999 of U.S. Army Soldiers. The data consisted of 126,312 enlisted soldiers from the 1979 cohort. The first hypothesis tested if different economic factors impacted enlisted retention rates over time. A second hypothesis tested if significant world events can be detected through a time series analysis by examining the occurrence of either pulse or level shifts. The results support both hypotheses and indicate future studies involving time series analysis with retention data would be useful in building more accurate retention models for U.S. Army enlisted soldiers.

**THE INFLUENCE OF ITEM WORDING ON QUESTIONNAIRE SCALE SCORES.** Nicole Anne Benn & Terry D. Dickinson, Department of Psychology, Old Dominion University, Norfolk, VA, 23529. Psychological researchers need methods for obtaining accurate responses from participants when using questionnaire data. The current study looked at the influence of negatively worded scale items on the overall scale. The NEO-PI was used to test the impact of negatively worded items. The NEO-PI was transformed to create four altered versions; each version contained differing amounts of negatively worded items. More than 1,100 participants were used in this study, with approximately 230 people in each subgroup. Confirmatory factor analysis, along with various goodness-of-fit measures, was used to test the results. Results show that negative wording affects the measurement properties of scales. Implications, limitations and ideas for future research are addressed.

**DIFFERENTIATION OF SELF AND INDIVIDUATION IN COLLEGE STUDENTS.** D. S. Vick, Virginia Consortium Program in Clinical Psychology Virginia Beach, VA 23462 & J. A. Morrow, Old Dominion Univ., Norfolk, Va. 23529-0267. This study explored the relationship between Bowen's (1978) construct of differentiation of self and Erikson's construct of individuation (1963) for traditional aged college students. Using non-probability sampling, the study included an ethnically diverse population of 84 college freshmen through seniors from a large, public southeastern university. The Differentiation of Self Inventory - DSI (Skowron, 2001) and the Psychological Separation Inventory - PSI (Hoffman, 1984), revealed a significant gender difference for Emotional Cutoff. No significant differences were revealed in looking at the DSI and PSI by class year. Significant negative correlations were identified between three DSI factors and the PSI factor Conflictual Independence.

**ETHNIC DIFFERENCES IN SIBLING RELATIONSHIPS USING THE RSRI.** Denise L. Miles & Jennifer Anne Morrow, Dept. of Psychology, Virginia Consortium Program in Clinical Psychology & Old Dominion University, Norfolk, VA 23529. Gaining understanding into the dynamics and ethnic differences in sibling relationships in young adulthood is beneficial in such realms as support networks, family therapy and coping strategies. This study modified the Sibling Relationship Inventory (RSRI) and examined the three specific factors of Affection, Hostility and Rivalry in young adult sibling relationships. In addition to testing the RSRI's reliability, it was compared to The Adult Sibling Relationship Questionnaire (ASRQ), which had the three comparable factors of Warmth, Conflict and Rivalry. One hundred and forty under-graduates (66 African American and 74 Caucasian) completed both surveys. Statistics indicated that the RSRI is an adequate measure of sibling relationships in young adulthood and that its relationship with the ASQR was such that Affection/Warmth and Hostility/Conflict had a significant positive correlation. This study also



examined ethnic differences on the three factors used in each scale. The study showed that Caucasian and African American young adults perceive their sibling relationships similarly over all the factors.

**STUDY STRATEGY USE IN RELATION TO TEST ITEM ASSESSMENT.** S. A. Bass, E. M. Justice & L. Briggs, Department of Psychology, Old Dominion University, Norfolk, VA 23508. Past research has illustrated the notion that successful educational outcomes are, for the most part, dependent upon how students process information as well as how students allocate their study time. The present research investigated whether a relationship exists between the types of study activities that students employ (i.e., strategy use) and their performance on different types of test questions. The degree to which students engage in different kinds of study activities was assessed using Form R of the Study Activity Survey (SAS-R). SAS-R scores were correlated with the percentage correct on different types of test questions. Researchers also attempted to reliably code multiple choice exam items according to the categories in the revised version of Bloom's taxonomy. Through use of these measures, the current study attempted to establish a relationship between different types of strategy use in conjunction with different types of questions. Correlations among SAS-R subscales, student demographics, and overall course performance indicated significant relationships between these constructs. Results of three stepwise multiple regression analyses indicated the subscales of Hyperprocessing and Self-Evaluation of Cognitive Ability to be significant predictors of overall course performance. Limitations and implications of the current research were addressed. It was concluded that the revised version of Bloom's taxonomy was insufficiently reliable in terms of classification of test items. Researchers further concluded that selective and self-monitoring study strategies appear to best predict overall course performance.

**DEVELOPMENTAL CORRESPONDENCE OF THEORY OF MIND AND INTENTIONAL STRATEGY USE.** Corrine E. Spiess & Elaine M. Justice, Dept. of Psychology, Old Dominion University, Norfolk, VA 23529. Children's understanding of intentions and strategy use was examined through interviewing 33 children enrolled at the Old Dominion University Child Development Center. Children of 4- and 5-years of age were told 8 stories and answered questions pertaining to the beliefs, desires, intentions, and surprise of characters in each story. Four intention and four strategy stories were narrated to each child. The results indicated no significant distinctions between genders or between age groups. However, a significant difference was discovered between the types of stories, with strategy stories easier for participants to understand.

**THE IMPACT OF PERSONALITY VARIABLES ON ACADEMIC PERFORMANCE.** Elizabeth Schmidt & Alice Alexander, Dept of Psych., Old Dominion University., Norfolk, Va. 23529. Previous research has demonstrated the existence of a relationship between personality variables and academic performance. However, recent research has revealed more consistent findings about this relationship. The current study examined the relationship between the Big Five personality variables (Extraversion, Conscientiousness, Agreeableness, Neuroticism, Openness to Experience) and academic performance (cumulative GPA, major GPA, and Final Introductory Psychology (FIP) course grade). Ninety-nine participants (84 females and 15 males) were recruited from various undergraduate psychology courses and asked to anonymously complete the 50-item personality inventory (IPIP) and to self-report their academic performance information. Bivariate correlations, one-way ANOVA's, and multiple regressions revealed that conscientiousness, openness to experience and neuroticism (lower levels of emotional stability) related the most to academic performance.

**COGNITIVE PRECURSORS TO SCIENCE COMPREHENSION.** Kimberly G. Cottrell & Danielle S. McNamara, Dept. of Psychology, Old Dominion University, Norfolk, VA 23529. Cognitive factors such as reading ability, prior domain knowledge, and reading strategy knowledge were examined to determine their ability to predict comprehension of a science text and course performance. The sample consisted of 144 undergraduate students enrolled in an introductory

psychology course at Old Dominion University. Science text comprehension was significantly predicted by both prior knowledge ( $\beta=.27$ ,  $sr_i^2=.04$ ) and reading ability ( $\beta=.41$ ,  $sr_i^2=.10$ ). Better readers and students with more knowledge about psychology answered more comprehension questions correctly. Exam performance was significantly predicted by prior domain knowledge ( $\beta=.48$ ,  $sr_i^2=.14$ ). Students with more knowledge about psychology before the course began obtained higher exam scores. The metacognitive reading strategy of drawing from prior knowledge enhanced science comprehension whereas previewing, purpose setting, and self-questioning enhanced the average of exams. Greater prior domain knowledge provided no benefit for students who did not use certain types of metacognitive reading strategies. Also, the tendency to use previewing strategies only benefited students if they possessed sufficient prior domain knowledge.

THE EFFECTS OF COMMUNICATION AND AFFECTIVE RESPONSE ON HUMAN COGNITION. Laura L. Nichols & Jeffrey A. Gibbons, Dept. of Psych., Christopher Newport Univ., Newport News, VA 23606. This study assessed 5 communication modes across cognition and affect in university-level students. The goal was to make covert thoughts and feelings apparent to the conscious mind of the individual, and to increase validity of response in relation to mood. Participants were placed in 1 of 5 groups: drawing, writing, psychodrama, symbolic interpretation, and reading. Participants completed a survey measuring levels of depression, stress, and anxiety before and after the specified communication technique. As predicted, scores of participants in all 5 groups differed across the pre and post survey. However, only the scores obtained from writing, reading, and symbolism groups were significantly different across pre and post surveys. Results of this study suggest that communication techniques such as writing, reading, and symbolic interpretation are beneficial to the individual in facilitation of valid response in relation to mood.

RAT PERFORMANCE IN THE RADIAL-ARM MAZE AFTER EXPOSURE TO *PFIESTERIA PISCICIDA* TOXIN AND SCOPOLAMINE ADMINISTRATION. Brian Parris<sup>1</sup>, Perry M. Duncan<sup>1</sup>, Brian Dyer<sup>2</sup>, and Howard Marshall<sup>2</sup>, <sup>1</sup>Department of Psychology, and <sup>2</sup>Department of Biological Sciences, Old Dominion University. *Pfiesteria piscicida* (pf) is a toxic dinoflagellate, and these experiments were examinations of the cognitive deficit produced in rats after exposure to the toxin from this marine micro-organism. Rats were trained in an eight-armed radial-arm maze (RAM), which tests working memory. Two dependent variables were measured: total time to complete the RAM, and total errors per trial. Experimental-group rats were injected subcutaneously with filtered water containing pf toxin, and controls with non-toxic filtrate. In the first experiment pf-exposed rats learned the RAM more slowly than did controls. In the second experiment rats were trained on the RAM first and then injected with pf filtrate. After learning the RAM, rats in both experiments were injected with scopolamine, an anticholinergic drug which impairs memory. In each experiment the pf-exposed rats were much more vulnerable to the drug-produced impairment as indicated by both dependent variables. The pf-rats required more time to complete the RAM, and made more errors. These results demonstrate that the pf toxin impairs some aspect of cognitive ability and increases vulnerability to scopolamine.

THE EFFECT OF INSULIN-INDUCED HYPOGLYCEMIA ON RAT PERFORMANCE IN THE DELAYED RADIAL-ARMED MAZE. Juan Constantine & Perry Duncan, Department of Psychology, Old Dominion University, Norfolk VA. Hypoglycemia (HG) causes various types of cognitive impairment, including memory failures, in humans. The purpose of this study was to investigate the possible effects of HG on reference memory in rats trained on the delayed radial arm maze (RAM), a procedure which allows separate testing of impairment of acquisition and retrieval of memory. Rats were trained to complete the RAM with a two-hour delay between the initial and the final phases of each trial. Insulin at 2 units/kg was administered before either the initial, the final, or both RAM phases, and each treatment's effect was compared with performance in the non-drugged control condition. HG levels of between 30 and 40 mg/deciliter (about 65% of normal blood-glucose levels) occurred 20 minutes after insulin injection. The first test for HG effect on retrieval

indicated a significant ( $p < .01$ ) memory impairment, but subsequent tests found no impairment. The lack of persistent memory impairment may be a result of behavioral tolerance to HG. HG during the initial RAM phase indicated no impairment, as did HG during both RAM phases. These results of no ongoing memory impairment may be due to relatively mild HG levels, or to this specific procedure for testing memory.

**POSITIVE INFLUENCES IN DEALING WITH ADVERSITY: A QUALITATIVE INVESTIGATION OF TRAUMA SURVIVORS.** Jennifer Ann Morrow & Sharon Clayman, Dept. of Psychology, Old Dominion University, Norfolk, VA 23529. Many children, while growing up, have been victims of trauma; trauma such as physical, psychological, and sexual abuse, as well as general neglect and witnessing violence between adults in the home. Positive influences that children experience while growing up can have a positive impact on their adulthood functioning. Unstructured, open-ended interviewing was used to ascertain trauma survivors' positive influences that helped them successfully overcome their traumatic experiences. Sixteen female childhood trauma survivors were interviewed for this study. Positive influences that many had in common were: spirituality or faith in a higher power, self-determination, having supportive others around them, and participating in sports or the creative arts. Future research should look at how one can instill these positive influences in young adults who have been traumatized.

**RELATIONSHIP BETWEEN COMMITMENT TO EXERCISE AND ANXIETY IN AMATEUR EXERCISERS.** Gabriela Dye & Jennifer Ann Morrow, Dept. of Psychology, Old Dominion University, Norfolk, VA 23529. This study was designed to assess the relationship between commitment to exercise and levels of anxiety in recreational exercisers. One hundred and seventy two participants, 138 University students and 34 health club members participated in this study. Of the 172 participants, 116 were females and 54 were males (age range: 18 to 66 years old). The results did not show a significant relationship between commitment to exercise and anxiety. Anxiety and frequency of exercise was negatively correlated. Results showed that males exercise more frequently, duration of exercise is longer, and they are more committed to exercise than females. Health club members exercise more frequently, duration of exercise is longer, and they are more committed to exercise than college students. Some of the limitations of the study were: small sample size from the health club, most participants engaged in moderate levels of exercise, the research did not focus on individuals who only participate in one type of exercise activity, and the anxiety scale used in this study measured general anxiety and not exercise anxiety. Future research should look at perceived fitness and satisfaction with ones' body and their interaction with commitment to exercise and anxiety.

**DISPLAY PARAMETERS AFFECTING PERCEIVED URGENCY AND ANNOYANCE IN VERBAL WARNING SIGNALS.** Sarah A. Matthews & Carryl Baldwin, Ph. D., Dept. of Psychology, Old Dominion Univ., Norfolk, Va 23529. Technology is advancing at a rapid pace bringing new advances in automotive systems including computerized collision avoidance systems, in-vehicle navigational displays and motorist advisory systems. Already common in some high-end cars, these warnings will better assist drivers in situations involving potential collisions, heavy traffic, adverse weather, and other complex driving situations. One new advanced system with the potential to dramatically increase motorist safety the in-vehicle collision avoidance systems (CAS). CA systems will ultimately assist drivers and help them to drive more cautiously. This study examines the influence of different signal words and the auditory presentation levels used to present these warnings on overall annoyance and perceived urgency. Four signal words (caution, danger, warning, and notice) were used in combination with a variety of CA messages. Results indicate that both signal word and presentation levels significantly impact the perceived urgency of the verbal messages.

## Statistics

A NOTE ON THE ESTIMATION OF AUTOCORRELATION IN REPEATED MEASUREMENTS. N. Rao Chaganty & Genming Shi, Dept. of Math. and Stat., Old Dominion University., Norfolk, Va 23529. In the analysis of repeated measurements with autocorrelation, popular methods of estimation are the maximum likelihood method and the method of moments used for normal and non-normal data, respectively. An alternative is the quasi-least squares method of estimation. This method, based on the principle of generalized least squares, does not make any distributional assumptions and differs from the two popular methods in the estimation of the autocorrelation. In this paper we study the large sample properties of the quasi-least squares estimates. Using the asymptotic relative efficiency criterion, for normally distributed measurements we show that the quasi-least squares estimates are better than the moment estimates and are good competitors to the maximum likelihood estimates. If normality is not tenable, the quasi-least squares estimates could be used as an alternative to the maximum likelihood and the moment estimates. (Supported partially by a grant from the US Army research office.).

SOME OPTIMALITY OF ROW-COLUMN DESIGNS. Siriluck Jermjitpornchai, Department of Mathematics and Statistics, Old Dominion University, Norfolk, Virginia 23529 & John P. Morgan, Department of Statistics, Virginia Polytechnic and State University, Blacksburg, Virginia 24061. This work uses an infinite series of nonbinary, unequally replicated E- and MV-optimal block designs from a result of Morgan and Srivastav (1999). These designs are considered to construct E- and MV-optimal row-column designs in an experimental setting where  $v$  treatments are arranged in  $bk$  experimental units being in an array consisting of  $k$  rows and  $b$  columns. An example is demonstrated

THE EFFECT OF MISCLASSIFICATION ON CLINICAL TRIALS. Cynthia S. Cors, Department of Biostatistics, Virginia Commonwealth University. Misclassification is examined as it pertains to categorical response variables and is defined as classifying a subject to an incorrect category. Simulation studies were used to investigate the changes in power at different levels of treatment improvement (0%, 15%, and 25%), combined with different levels of misclassification (0%, 10%, and 20%), with 2, 4, and 8 outcome categories. The power function indicated that power increases when there are no misclassifications and tends to be higher with the smallest number of outcome categories. The following three categorical distributions were examined: bell-shaped, uniform, and U-shaped. This is important in the design of clinical trials because reducing the number of outcome categories should also decrease the potential for misclassification, thereby maximizing power for the study.

ADAPTIVE FOURIER ANALYSIS FOR UNEQUALLY-SPACED TIME SERIES DATA. Hong Liang & Robert V. Foutz, Department of Statistics, Virginia Polytechnic Institute and State University. Fourier, Walsh-Fourier, and wavelet analysis have been used in time series. Fourier analysis might be misleading when the periodic components are not sinusoidal. Walsh-Fourier analysis is difficult to interpret since Walsh functions are not periodic. Wavelet analysis also has flaws. It gives no exact meaning to the concept of frequency. All three methods require equally-spaced observations. In this paper, by using a sequence of periodic step functions, a new analysis method, adaptive Fourier analysis, and its theory are presented. These can be applied to time series where patterns may take general periodic shapes that include sinusoids as special cases. Most importantly, the resulting adaptive Fourier analysis does not require equally-spaced time series observations.

**VIRGINIA JUNIOR ACADEMY OF SCIENCE  
AWARDS****AGRICULTURAL AND ANIMAL SCIENCE**

Honorable Mention: JULIA K. STOLBERG AND RACHEL E. KALLEM  
Yorktown High School  
Third Place: LAURA L. SPRAGUE  
Tuckahoe Middle School  
Second Place: KATHERINE A. HOWELL  
James River High School  
First Place: ZACHARY J. CAPPELLO  
Lee-Davis High School

**ANIMAL BEHAVIOR (ETHOLOGY)**

Honorable Mention: TRACEY K. GRIFFITH  
Goochland High School  
Honorable Mention: MARY BETH S. JOHNSON  
Tuckahoe Middle School  
Third Place: CAROLINE K. HEGGIE  
Tuckahoe Middle School  
Second Place: VALERIE M. HOANG  
Mills E. Godwin High School  
First Place: KARA L. CAPELLI  
Atlee High School

**BOTANY A**

Honorable Mention: ROGER B. ARNOLD, JR.  
Tuckahoe Middle School  
Honorable Mention: NATALIE T. CLAVON  
Central Virginia Governor's School  
Third Place: KATHERINE M. FOSTER  
Yorktown High School  
Second Place: WREN ELHAI  
Maggie L. Walker Governor's School  
First Place: TREVOR E. CORBIN  
Harry F. Byrd Middle School

## BOTANY B

- Honorable Mention: SEAN C. KITCHEN  
Tuckahoe Middle School
- Honorable Mention: THOMAS R. MALLORY  
Patrick Henry High School
- Honorable Mention: KATHERINE A. PUCKETT  
Manchester Middle School
- Third Place: SARAH K. GALLAGHER  
Central Virginia Governor's School
- Second Place: MAYA I. MACKRANDILAL  
Yorktown High School
- First Place: ROBERT L. GREEN  
Central Virginia Governor's School

## BOTANY C

- Honorable Mention: REBECCA E. SLOANE  
Chickahominy Middle School
- Honorable Mention: EMILY W. WHITTET  
Tuckahoe Middle School
- Third Place: ELLEN A. YOUNG  
Maggie L. Walker Governor's School
- Second Place: KATHERINE A. RUBIDA  
Washington-Lee High School
- First Place: EMILY C. SMITH  
Southwest Virginia Governor's School

## CHEMISTRY A

- Honorable Mention: NICHOLAS M. BIRASA  
Swanson Middle School
- Honorable Mention: JAN MARIE HEWITT  
Summit Christian Academy
- Honorable Mention: SHELLY L. JONES  
Warwick High School
- Third Place: ANN C. KIRCHMAN  
Central Shenandoah Valley Governor's School
- Second Place: SCOTT W. DECKER  
Mathematics and Science High School at Clover Hill
- First Place: JONATHAN S. CHAN  
Mathematics and Science High School at Clover Hill

**CHEMISTRY B**

- Honorable Mention: ANTHONY P. LEONARD  
Southwest Virginia Governor's School
- Honorable Mention: SARAH MCCLENDON  
George H. Moody Middle School
- Honorable Mention: WYLIE B. PENNELL  
Warwick High School
- Third Place: JAMES V. PECK, IV  
Harry F. Byrd Middle School
- Second Place: DAVID MINASKANIAN  
Mills E. Godwin High School
- First Place: JASON A. NG  
Yorktown High School

**CHEMISTRY C**

- Honorable Mention: SARA J. RIPPEL  
James River High School
- Honorable Mention: SOHINI SIRCAR  
Harry F. Byrd Middle School
- Honorable Mention: KIMBERLY A. STAIB AND CHRISTINA H. WALES  
West Potomac High School
- Third Place: NIRAJA SATHYANARAYANAN  
Mills E. Godwin High School
- Second Place: M. HUNTER WITT  
Gildersleeve Middle School
- First Place: ROBERT L. THOMAS  
Yorktown High School

**COMPUTER SCIENCE**

- Honorable Mention: WASEEM S. DAHER  
Yorktown High School
- Honorable Mention: RYAN C. MCKENZIE  
Ocean Lakes High School
- Honorable Mention: TAUHIDUL HOGUE  
Yorktown High School
- Third Place: DREW G. STEPHENS  
Yorktown High School
- Second Place: MICHAEL J. DOBBS  
Yorktown High School
- First Place: MATTHEW S. BIGELOW  
Central Shenandoah Valley Governor's School

## CONSUMER SCIENCE A

- Honorable Mention: JUSTIN M. BRECKLEY AND ROBERT A. DORFMAN  
Gildersleeve Middle School
- Honorable Mention: MATTHEW R. CAPELLI  
Oak Knoll Middle School
- Honorable Mention: RITA L. DIGRAZIA AND JAIME A. GOLDBERG  
James River High School
- Third Place: SARAH M. COUSINS  
Maggie L. Walker Governor's School
- Second Place: VANESSA P. CRANDALL  
Central Shenandoah Valley Governor's School
- First Place: ABIGAIL V. BROWN AND MARISSA L. LEVENDIS  
Yorktown High School

## CONSUMER SCIENCE B

- Honorable Mention: ASAKO KUBOTA  
Cave Spring High School
- Honorable Mention: MARY E. GAYLE  
Chickahominy Middle School
- Honorable Mention: KRISTIN M. MELNYCZUK  
Central Shenandoah Valley Governor's School
- Third Place: EMILY M. GREENE  
Central Shenandoah Valley Governor's School
- Second Place: ERIK W. HAUG  
Central Shenandoah Valley Governor's School
- First Place: MICHAEL R. LANDIS  
Chickahominy Middle School

## CONSUMER SCIENCE C

- Honorable Mention: SARAH K. PETERS  
James River High School
- Honorable Mention: HOLLI S. REEVES  
Mills E. Godwin High School
- Honorable Mention: OLGA R. SHERMAN  
Mills E. Godwin High School
- Third Place: ARJUN MUTHU  
Maggie L. Walker Governor's School
- Second Place: BRITTANY L. VASCIK  
Cave Spring High School
- First Place: MATTHEW S. PELTZ  
Gildersleeve Middle School



**EARTH AND SPACE SCIENCE**

- Honorable Mention: RACHEL L. BEATON  
Central Virginia Governor's School
- Honorable Mention: ANDREW V. GARDEN  
Woodrow Wilson Middle School
- Honorable Mention: KYLE G. PRESSEL  
Mills E. Godwin High School
- Third Place: SHELLY T. DEVEREAUX  
Southwest Virginia Governor's School
- Second Place: ANEESH K. VENKAT  
New Horizons Governor's School
- First Place: LAURA G. GAMSE  
Yorktown High School

**ENGINEERING A**

- Honorable Mention: COREY A. BARNES  
Central Virginia Governor's School
- Honorable Mention: IAN K. BARNES  
Gildersleeve Middle School
- Honorable Mention: JAMES R. DAVIS  
Central Shenandoah Valley Governor's School
- Third Place: JEREMY S. GRABOYES  
Maggie L. Walker Governor's School
- Second Place: BETH R. ENGLAND  
Williamsburg Middle School
- First Place: CHRISTY E. CLAPP  
Lloyd C. Bird High School

**ENGINEERING B**

- Honorable Mention MATTHEW T. ROY  
Mathematics and Science High School at Clover Hill
- Honorable Mention: BRANDON R. SMITH  
Lloyd C. Bird High School
- Honorable Mention: CHANTE' M. STUBBS  
Crittenden Middle School
- Third Place: BRANDON J. MURRILL  
Mills E. Godwin High School
- Second Place: GEORGE S. TARASIDIS  
James River High School
- First Place THOMAS MORIE  
Williamsburg Middle School

## ENVIRONMENTAL SCIENCE A

- Honorable Mention: JEAN M. FOLSOM  
Yorktown High School
- Third Place: RAVI DEEPAK  
Poquoson High School
- Second Place: MATTHEW G. COLLINS  
Cave Spring High School
- First Place: CURTIS C. COPELAND  
Gloucester High School

## ENVIRONMENTAL SCIENCE B

- Honorable Mention: AUSTIN K. GIBSON  
Central Shenandoah Valley Governor's School
- Honorable Mention: MYLES K. GLANCY  
Mills E. Godwin High School
- Honorable Mention: MRINAL R. MENON  
Maggie L. Walker Governor's School
- Third Place: JENNA L. MAXFIELD  
Atlee High School
- Second Place: JOANN E. KELLY  
Gloucester High School
- First Place: RUTH A. HALL  
Mills E. Godwin High School

## ENVIRONMENTAL SCIENCE C

- Honorable Mention: ALYSSA J. MULLINS  
Manchester Middle School
- Honorable Mention: JESSICA L. RIPLEY  
Chesapeake Bay Governor's School
- Honorable Mention: KRISTA L. ROOP  
Mills E. Godwin High School
- Third Place: NATHAN H. RAINES  
Yorktown High School
- Second Place: ANDREW P. SCHUKMAN  
Mills E. Godwin High School
- First Place: JONATHAN D. RAMALEY  
Warwick High School

**ENVIRONMENTAL SCIENCE D**

- Honorable Mention: ERICA D. SIEGMUND  
Maggie L. Walker Governor's School
- Honorable Mention: CHRISTOPHER R. SIMPSON  
Central Virginia Governor's School
- Honorable Mention: KAMBER VITTORI AND MARGARET SAUNDERS  
Warwick High School
- Third Place: ANDREW R. SPOTTS  
Mills E. Godwin High School
- Second Place: ANEESH K. VENKAT  
New Horizons Governor's School
- First Place: MATTHEW E. WALKER  
Mills E. Godwin High School

**GENETICS AND CELLULAR BIOLOGY**

- Honorable Mention: NISHA F. LIGON  
Maggie L. Walker Governor's School
- Honorable Mention: NAOMI E. ROSS  
Yorktown High School
- Honorable Mention: LANA M. SHAHMORADIAN  
Mills E. Godwin High School
- Third Place: KELSEY L. TAYLOR  
Yorktown High School
- Second Place: DANIEL B. FISHER  
Stonewall Jackson Middle School
- First Place: MARISSA R. KESSLER  
Mills E. Godwin High School

**MATHEMATICS**

- Honorable Mention: AUSTIN P. CRISPENS  
Mathematics and Science High School at Clover Hill
- Third Place: EMILY S. WREN  
Yorktown High School
- Second Place: ANKIT JAIN  
Mills E. Godwin High School
- First Place: LYRIC P. DOSHI AND JOSEPH E. GONZALEZ  
Maggie L. Walker Governor's School

## MEDICINE AND HEALTH A

- Honorable Mention: SARAH A. BENSON  
Thomas Jefferson High School for Science and Technology
- Honorable Mention: ERICA A. BROTZMAN  
Harry F. Byrd Middle School
- Honorable Mention: SOLON CHOI  
Mathematics and Science High School at Clover Hill
- Third Place: CAREY C. ARCHER  
Mills E. Godwin High School
- Second Place: VICTORIA L. CHIOU  
Maggie L. Walker Governor's School
- First Place: DAVID K. BAUMGARTEN  
Collegiate School

## MEDICINE AND HEALTH B

- Honorable Mention: ARIEL R. GERBER  
Mills E. Godwin High School
- Honorable Mention: KATELYN R. GOOD  
Manchester Middle School
- Honorable Mention: JAMES N-HOKE  
Maggie L. Walker Governor's School
- Third Place: LINDA K. EVANS  
Central Virginia Governor's School
- Second Place: LINDSEY N. JONES  
Central Virginia Governor's School
- First Place: SVETLANA Y. LANTSMAN  
Mills E. Godwin High School

## MEDICINE AND HEALTH C

- Honorable Mention: JULIE C. NOBLE  
Yorktown High School
- Honorable Mention: GAURI R. RAVAL  
Mills E. Godwin High School
- Honorable Mention: NAMRATA VERMA  
New Horizons Governor's School
- Third Place: SURAVI SIRCAR  
Mills E. Godwin High School
- Second Place: MICHELLE S. OH  
Thomas Jefferson High School for Science and Technology
- First Place: ONO U. NSEYO  
Mills E. Godwin High School

**MICROBIOLOGY A**

- Honorable Mention: SARAH E. BOMAN  
Central Virginia Governor's School
- Honorable Mention: CONOR J. FRACKLETON  
Central Virginia Governor's School
- Honorable Mention: JEREMY S. GRABOYES  
Maggie L. Walker Governor's School
- Third Place: XAVIER W. BELCHER  
Huguenot High School
- Second Place: LAURA E. BLACK  
Yorktown High School
- First Place: PETER D. HOGGE  
Gloucester High School

**MICROBIOLOGY B**

- Honorable Mention: DAVID H. KELLAM  
Ocean Lakes High School
- Honorable Mention: KEVIN M. MALLON AND SEAMUS D. LYNCH  
Washington-Lee High School
- Honorable Mention: RAYMOND L. PLAMER, JR.  
Varina High School
- Third Place: JASMINE C. MAJOR  
Hermitage High School
- Second Place: DEVON M. MILLER  
Tuckahoe Middle School
- First Place: NEEL K. RAI  
Maggie L. Walker Governor's School

**PHYSICS A**

- Honorable Mention: ERIC J.P. ACHESON AND VICTOR A. DROPIK  
Swanson Middle School
- Honorable Mention: BENJAMIN P. CERTNER  
Tuckahoe Middle School
- Honorable Mention: REBECCA L. EFFRON  
Harry F. Byrd Middle School
- Third Place: BAIJU D. BHATT  
Poquoson High School
- Second Place: ANDREA R. ENGLAND  
Southwest Virginia Governor's School
- First Place: MELISSA A. DISKIN  
Gildersleeve Middle School

## PHYSICS B

- Honorable Mention: PETER L. HARLAN  
Yorktown High School
- Honorable Mention: CHRIS G. HINCKER  
Woodrow Wilson Middle School
- Honorable Mention: KELLY M. O'BRIANT  
Swanson Middle School
- Third Place: DANIEL P. HARLAN  
Yorktown High School
- Second Place: DEREK S.Y. MILLER  
Maggie L. Walker Governor's School
- First Place: GRACE C. HOU  
New Horizons Governor's School

## PHYSICS C

- Honorable Mention: JACQUELINE K. RODERICK  
Warwick High School
- Honorable Mention: BARBARA J. WARD  
Mathematics and Science High School at Clover Hill
- Honorable Mention: RACHEL E. WATSON  
Gildersleeve Middle School
- Third Place: ANNA M. PAVLUK  
Yorktown High School
- Second Place: STEPHEN P. SOJKA  
Mills E. Godwin High School
- First Place: XUN Y. ZHU  
Manchester Middle School

## PSYCHOLOGY - GENERAL

- Honorable Mention: COLLEEN E. KARAFFA  
Central Shenandoah Valley Governor's School
- Honorable Mention: LARISSA C. MOUNT  
Washington-Lee High School
- Honorable Mention: KATHLEEN NORLAND  
Yorktown High School
- Third Place: TRACY L. JENNINGS  
George H. Moody Middle School
- Second Place: KYLE H. DALY  
Central Virginia Governor's School
- First Place: CAROLINE A. DULANEY  
H.B. Woodlawn

PSYCHOLOGY - LEARNING & PERCEPTION A

- Honorable Mention: MEREDITH L. COCKS  
Yorktown High School
- Honorable Mention: RACHEL V. KLING  
Tuckahoe Middle School
- Third Place: VICTORIA CHILDRESS  
Mills E. Godwin High School
- Second Place: MELANIE A. DAVIS  
Central Virginia Governor's School
- First Place: JULIE M. KRAFT  
Stonewall Jackson Middle School

PSYCHOLOGY - LEARNING & PERCEPTION B

- Honorable Mention: BRITTANY N. NEWELL  
J.R. Tucker High School
- Honorable Mention: JANANI SUNDAR  
Mills E. Godwin High School
- Honorable Mention: JOHN R. WALK  
Tuckahoe Middle School
- Third Place: KRISTIN E. PANKE AND JENNA R. ALLEN  
Yorktown High School
- Second Place: AMANDA A. TRAYNHAM  
Central Shenandoah Valley Governor's School
- First Place: WILLIAM L. TUFTS  
Central Shenandoah Valley Governor's School

PSYCHOLOGY - SOCIAL

- Honorable Mention: JEREMY D. KIDD  
Southwest Virginia Governor's School
- Honorable Mention: NICOLE A. THOMAS AND MAEGEN C. SMITH  
Atlee High School
- Honorable Mention: SARAH E. ULMER  
George H. Moody Middle School
- Third Place: ALEXANDRA E. JATRAS  
Yorktown High School
- Second Place: RACHEL L. CARTER  
Tuckahoe Middle School
- First Place: THOMAS A. BERENATO  
Blacksburg High School

## STATISTICS

- Third Place: CHRISTOPHER U. EMBA  
Mills E. Godwin High School
- Second Place: KATHERINE MURTHA  
Yorktown High School
- First Place: VICTOR D. FEDOROV  
Mills E. Godwin High School

## ZOOLOGY A

- Honorable Mention: EMILY K. HESALTINE  
Mills E. Godwin High School
- Honorable Mention: THOMAS D. JOHNSON  
Mary L. Passage Middle School
- Third Place: SAVANNAH M. FETTEROLF AND MEAGAN WILKINS  
Hayfield High School
- Second Place: CHARLES S. JOHNSON  
Southwest Virginia Governor's School
- First Place: DAVID A. GIBBS  
H.B. Woodlawn

## ZOOLOGY B

- Honorable Mention: DAVID J. NYCZEPIR  
Manchester Middle School
- Honorable Mention: ERIN B. STRANG  
Lee-Davis High School
- Third Place: LOUISE A. MONTGOMERY  
Yorktown High School
- Second Place: CHIDI J. ONYESO  
Mills E. Godwin High School
- First Place: MAEGAN J. MICHAEL  
Mills E. Godwin High School

## SPECIAL AWARDS

- Botany Section Award, given by the Botany Section of the VAS, to the best paper on a botanical subject. (\$200.00)
- ROBERT L. GREEN  
Central Virginia Governor's School



VJAS Neuroscience Awards supported by the Virginia Neurological Society and the Auxiliary of the Virginia Neurological Society are given to up to four out-standing papers in the field of neuroscience (\$100.00 each).

VICTORIA L. CHIOU

Maggie L. Walker Governor's School

Speleological Society Award given to the best paper addressing karst or topics related to speleology given by the Richmond Area Speleological Society. (\$500.00)

PETER GRIMALDI AND PATRICK RAINEY

Yorktown High School

Mathematics Award for the paper that evidences the most significant contribution in the field of Mathematics. (\$200.00)

LYRIC P. DOSHI AND JOSEPH GONZALES

Maggie L. Walker Governor's School

Statistics Award for the paper that evidences the most significant contribution in the field of Mathematics. (\$200.00)

VICTOR D. FEDOROV

Mills E. Godwin High School

Smith Shadomy Infectious Disease Award in honor and memory of Dr. Smith Shadomy given by the Virginia Chapter of the National Foundation of Infectious Diseases. (\$50.00)

NEEL RAI

Maggie L. Walker Governor's School

Roscoe Hughes Award for the best paper in the field of Genetics. (\$200.00)

KELSEY L. TAYLOR

Yorktown High School

Rodney C. Berry Chemistry Award for the paper that evidences the most significant contribution in the field of chemistry. (\$400.00)

JASON A. NG

Yorktown High School

The Dr. and Mrs. Preston H. Leake Award in Applied Chemistry will be given to the author of a research paper which best exemplifies how chemicals, chemical principles, or chemistry have been used, are used, or might be used to enhance or even to save life. (\$200.00)

GAURI RAVAL

Mills E. Godwin High School

Russell J. Rowlett Award for the Best Research Paper of the Year. (\$300.00)

DAVID K. BAUMGARTEN

Collegiate School

The Virginia Psychological Foundation Meritorious Research Awards recognize outstanding presentations of research in the various fields of psychology. Each award includes a prize of \$100.00.

CAROLINE DULANEY

H.B. Woodlawn

JULIE M. KRAFT

Stonewall Jackson Middle School

WILLIAM L. TUFTS

Central Shenandoah Valley Governor's School

THOMAS A. BERENATO

Blacksburg High School

Virginia Sea Grant College Program Award is given by the Virginia Sea Grant College Program for outstanding marine or coastal research. (\$125.00)

JOANN E. KELLY

Gloucester High School

American Cancer Society Award - This award is to recognize outstanding science papers related to cancer research. These awards are provided by the American Cancer Society (Virginia Council).

Honorable Mention - (\$50)

GAURI RAVAL

Mills E. Godwin High School

Third Place - (\$100)

PETER GRIMALDI AND PATRICK RAINEY

Yorktown High School

First Place - (\$400)

KONSTANTIN Y. LANTSMAN

Maggie L. Walker Governor's School

The Gamma Sigma Delta Award (Agriculture). Presented by the VPI & SU Chapter of the Honor Society of Agriculture. This award of \$100 is presented in recognition of excellence in research dealing with application of new technologies and/or concepts in agriculture forestry, or veterinary medicine. (\$100.00)

ZACHARY J. CAPPELLO

Lee-Davis High School

Dominion - W.W. Berry Award. This award is given by Dominion Virginia Power in honor of Mr. W. W. Berry who was a past Chairman of the Board of VA Power. This award of a \$500.00 Savings Bond will be presented to the best engineering paper.

CHRISTY E. CLAPP

Lloyd C. Bird High School

The Joyce K. Peterson Award is presented for the outstanding paper by a middle school student. It is presented in honor of Mrs. Joyce K. Peterson who has been an outstanding teacher in the Arlington County Schools. (\$75)

KELLY O'BRIANT  
Swanson Middle School

The Ann M. Hancock Award - This award is given to the best paper in genetics and is given in memory of Anne Hancock who retired from Patrick Henry High School in Hanover County and who gave many years of service to the Jr. Academy not only by teaching but also serving on the Jr. Academy Committee. Whitney Hancock, granddaughter of Ann Hancock, presented the award. (\$200)

MARISSA R. KESSLER  
Mills E. Godwin High School

Dorothy Knowlton Award - This award is given to the best paper in the Consumer Science section(s) and is given in honor of Dorothy Knowlton, former Science Coordinator of Arlington County Schools. (\$75)

JORDAN EFFRON  
Mills E. Godwin High School

VABE Award - This award is presented by the Virginia Association of Biology Educators and is given for outstanding research in the Zoology section(s). (\$50) This award presented by *Marion Lobstein*.

DAVID A. GIBBS  
H.B. Woodlawn  
MAEGAN J. MICHAEL  
Mills E. Godwin High School

Trip to AJAS - AAAS Meeting for two students and two alternates for presenting outstanding papers. The 1999 meeting will be held in Feb. in *Denver*.

Winner: ZACHARY CAPPELLO  
Lee-Davis High School

Winner: PETER HOGGE  
Gloucester High School

Alternate: JONATHAN RAMALEY  
Warwick High School

Alternate: THOMAS MORIE  
Williamsburg Middle School

Honorary Membership - AAAS given to two students.

WHIT SALIBA  
Patrick Henry High School  
BOB HSIA  
Mills E. Godwin High School

Honorary Membership - VAS given to a student.

ERIN FRACKLETON

University of Virginia

Bethel High School Scholarship - This \$1,000 Scholarship Award comes from the interest earned from a \$10,000 endowment contributed by the students of Bethel High School, Hampton, Va., over a two year period. Accompanying this scholarship is a rotating plaque to be displayed in the student's school for the next year.

This award is based on both the students presentation and paper.

MARISSA KESSLER

Mills E. Godwin High School

Frances and Sydney Lewis Environmental Scholarship: A \$14,000 scholarship (\$3,500 per year for four years) for the best effort by a student in grades 9 to 12 in the field of environmental science. This scholarship is in the name of Frances and Sydney Lewis and is given by the Virginia Environmental Endowment.

CURTIS C. COPELAND

Gloucester High School

E.C.L. Miller Science Teacher of the Year Award is given to an outstanding science teacher. An all-expense-paid trip to next year's AAAS which will be in *Denver*.

PATRICIA COLTRANE

Warwick High School

VJAS Distinguished Service Award, most prestigious award given by the VJAS, is presented to a person for exceptionally outstanding service to the VJAS.

JUDY UPCHURCH

President and officers election results

Secretary - Carrie DuLaney - H.B. Woodlawn

Vice President - Suravi Sirca - Godwin HS

President - Eric Nielsen - Maggie Walker Gov's School

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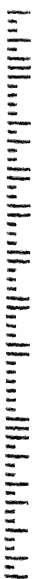
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## Chromium Tolerant Microbial Communities from the Chesapeake Bay Watershed

Kristine L. Lowe<sup>1</sup>, Ruth E. Fliflet<sup>1</sup>, Tony Ly<sup>1</sup>, Brenda J. Little<sup>2</sup>  
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Key Words: Chromium tolerance, *Shewanella*, contaminant metals,  
Chesapeake Bay

### ABSTRACT

Chromium tolerant bacteria were enumerated from portions of the Chesapeake Bay watershed and examined for their potential to reduce Cr(VI). Water and sediment samples were collected from various locations in Baltimore Harbor and Bear Creek, as well as Sandy Point State Park in Maryland and the Anacostia River in Washington, DC. Samples were spread onto agar plates with CrO<sub>4</sub><sup>2-</sup> (5 ppm) as the sole terminal electron acceptor. Plates were incubated anaerobically and colony forming units (CFU) enumerated. CFU arising on minimal-CrO<sub>4</sub><sup>2-</sup> medium ranged from 10<sup>3</sup>-10<sup>4</sup> mL<sup>-1</sup> or g<sup>-1</sup> and community estimates from sites in proximity to Baltimore City were approximately 6-30X higher than distal sites. Bacterial identification by BIOLOG<sup>TM</sup> or 16S rRNA sequencing indicated the presence of bacteria of the genera *Klebsiella*, *Pseudomonas*, *Burkholderia*, *Kluyvera* and others. Typical Cr(VI) reduction rates by these isolates were significantly lower than *Shewanella oneidensis*, a known metal-reducing bacterium. Results suggested that microbial communities in the Chesapeake Bay watershed, particularly in Baltimore Harbor and Bear Creek, had a high tolerance for Cr(VI) and/or could grow slowly with Cr(VI) as a terminal electron acceptor. However, the isolates did not rapidly degrade Cr(VI) in the laboratory.

### INTRODUCTION

The Chesapeake Bay is the largest estuary in the U.S. and is fed by a broad watershed that includes six states (New York, Pennsylvania, Delaware, Maryland, Virginia and West Virginia) and the District of Columbia, encompassing an area of approximately 12,000 km<sup>2</sup> (Pritchard and Schubel, 2001). Forests, cultivated and abandoned agricultural land, wetlands and residential areas surround the Bay and its adjacent watershed. It is home to a wide range of aquatic wildlife and has regional economic importance in the fishery and shipping industries (Lippson and Lippson, 1997). In addition, the Chesapeake Bay is a popular site for recreational boaters and tourists.

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As a result of past and recent human activities, pollutants and other contaminants (i.e., pesticides, herbicides, organophosphates, polychlorinated biphenyls [PCBs], petroleum products and heavy metals) have accumulated in the Bay (Lynch, 2001). Pollutants reach the Bay through river drainage, runoff and direct discharge (Curtin, 2001). One of the more problematic contaminants is chromium, which was mined north of the Bay in the 19th and 20th centuries. Chromium is an important industrial metal used in the manufacture of many diverse products, including ferrous and nonferrous alloys, paints, pigments, wood preservatives and corrosion inhibitors (Fendorf et al., 2000). Such manufacturing industries have operated in and around the Bay region during the past two centuries.

Chromium is a redox active transition metal with a wide range of possible oxidation states, although, only two (+6 and +3) are stable in the environment. It is a widespread contaminant in the environment and is recognized as a toxic substance and carcinogen (Kimbrough et al., 1999). Cr(VI) is highly water soluble and is easily transported through aquatic environments. In contrast, Cr(III) is much less soluble and precipitates as a hydroxide above pH 5.5. Due to its lower solubility, Cr(III) is considered less toxic and is, in fact, a necessary micronutrient for humans and other animals (Hamilton and Wetterhahn, 1987).

Chromium tolerance may occur by several potential mechanisms including plasmid-encoded resistance, transport mechanisms and reduction (Wang, 2000; Cervantes et al., 2001). Reduction of soluble (more toxic) Cr(VI) to less soluble (less toxic) Cr(III) is influenced by several factors (e.g., pH, temperature, redox potential) and can be mediated by various chemical species (i.e., Fe(II),  $S^{2-}$ ), some plants and several microorganisms (Fendorf et al., 2000; Lytle et al., 1998; Wang, 2000). A metal-reducing microorganism, *Shewanella oneidensis*, has been shown in laboratory experiments to reduce Cr(VI) at high rates (Daulton et al., 2001). Thus, one potential strategy for environmental Cr(VI) removal would be the addition of *S. oneidensis* into contaminated sites. However, it is not known at this time whether *S. oneidensis* can compete with native microflora at Cr(VI) contaminated sites. Therefore, a possible remediation plan would be to stimulate naturally-occurring Cr-tolerant and Cr(VI)-reducing bacteria (CRB) in contaminated environments by fertilization or other environmental manipulation. Alternatively, wastewater treatment schemes could be developed using naturally-occurring CRB in bioreactor systems. *In situ*, naturally-occurring CRB may have Cr(VI) reduction capabilities superior to those of *S. oneidensis*.

To assess the feasibility of such bioremediation strategies, we evaluated the prevalence of Cr-tolerant and other bacterial communities in the Chesapeake Bay watershed, which includes regions previously shown to contain high levels of contaminants including chromium (Baker et al., 1997). Environmental isolates were identified and tested for their ability to reduce Cr(VI) in the laboratory.

## METHODS

### Sampling Locations.

Surface water samples (top 2 cm) were collected from five sites in the Chesapeake Bay watershed on July 14, 2000 (Figure 1). They included: two sites, HP (39° 17' 08" N, 76° 36' 42" W) and FP (39° 16' 53" N, 76° 35' 33" W) in Baltimore City Harbor; one site, FM (39° 15' 46" N, 76° 34' 43" W), approximately 2 km downstream; and two sites distal to Baltimore. The distal sites included: one site approximately 40 km further

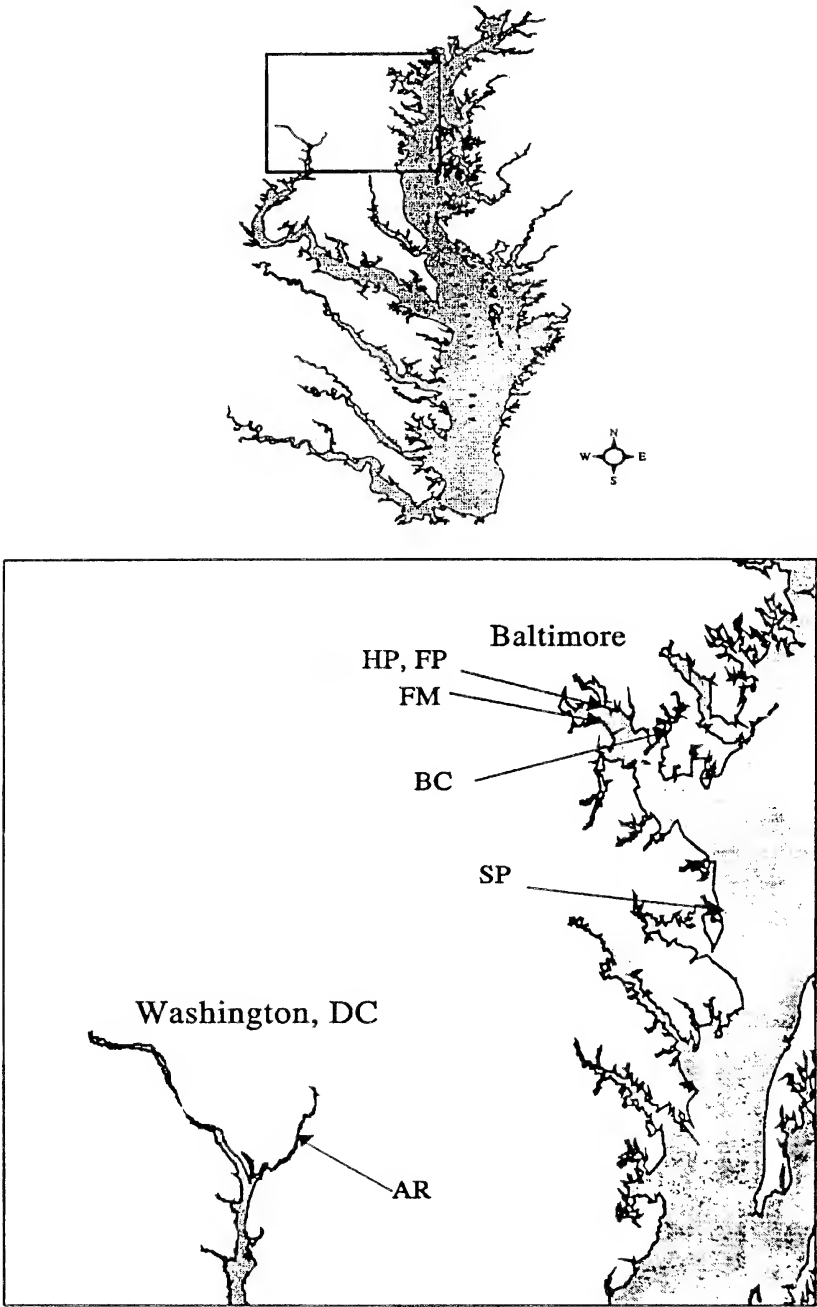


FIGURE 1. Map of sampling locations

downstream at Sandy Point State Park (SP; 39° 00' 44" N, 76° 23' 42" W) near Annapolis, Maryland and a second site in the northwest branch of the Anacostia River near Washington, DC (AR; 38° 53' 17" N, 76° 58' 04" W), approximately 60 km west of the Bay proper. Sediment samples were collected on July 3, 2001 from Bear Creek (BC; 39° 14' 41" N, 76° 29' 40" W), a tributary of the Patapsco River near Dundalk, Maryland. Bear Creek sediment samples were taken in a small channel that is fed by the main creek at high tide. Suction corers were used to remove 18-cm deep columns of sediment, which were stored on ice during transfer to the laboratory. The sediment cores were sectioned into 2-cm deep layers, each layer was homogenized and processed as described below. At all sites, water temperature was 26°C and salinity was approximately 1‰ (Pritchard and Schubel, 2001).

#### Sediment Chemical Analysis.

A portion of each 2-cm sediment sample was examined for the presence of chromium (Cr), iron (Fe), zinc (Zn) and nickel (Ni). Metals were extracted from sediment using the method of James et al. (1995). Briefly, 5 g of sediment were placed in sterile 125 mL flasks with 25 mL of a carbonate-hydroxide buffer (pH 13). The mixture was shaken until homogeneous and left standing for 1 h. The flasks were transferred to an 80°C water bath for 45 min and were mixed every 15 min. Due to evaporation, sterile distilled water was added as required to maintain a constant volume. After incubation, sediment slurries were transferred to sterile tubes and centrifuged for 30 min at 3,000 rpm. The supernatant was collected, filtered through a 0.2 µm filter and analyzed for heavy metals by Flame Atomic Absorption Spectroscopy (FAAS) using an air-acetylene flame (Model 906; GBC Scientific Equipment, Arlington Heights, Illinois). Standard curves were generated for each metal (Cr, Fe, Zn, and Ni) using purified standards. Sediment metal concentrations were estimated from plotted absorbance values on standard curves. The instrument automatically corrected for background interference by subtracting absorbance measurements from a deuterium lamp operating concurrently with the analyte light source.

#### Culture Incubations.

All incubations were performed in duplicate at 26°C. An aliquot (100 µL) was withdrawn from each water sample and inoculated into liquid enrichment cultures consisting of a defined minimal medium (Daulton et. al., 2001) supplemented with 18 mM lactate and 5 ppm Cr(VI) in the form of K<sub>2</sub>CrO<sub>4</sub> (Sigma, St. Louis, Missouri), as the sole terminal electron acceptor (medium hereafter abbreviated NS). Enrichment cultures were incubated anaerobically in glass canisters using an anaerobic gas generating system (BBL Gas Pak Plus; Becton Dickinson Co., Cockeysville, Maryland). After 10 days, 1 mL of each enrichment culture was removed, serially diluted and spread onto solid NS medium. Dilutions spread onto NS plates were immediately placed into anaerobic canisters at 26°C. After 3 weeks, the canisters were opened and colony forming units (CFU) per mL of original water sample estimated.

A 1 g sample from each homogenized sediment layer of Bear Creek was transferred to 9 mL of sterile saline, serially diluted and spread onto commercially-prepared agar media (Nutrient Agar [NA]; Difco Laboratories, Detroit, Michigan) supplemented with NaCl (1.5% w/v) and NS agar plates. One set of NA plates was incubated aerobically for 1 week. A second set of NA plates and the NS plates were placed in anaerobic

canisters at 26°C. After 3 weeks, the canisters were opened and CFU g<sup>-1</sup> wet sediment were enumerated.

CFU data were determined on NS and other plates by counting any visually-observed colonies arising on the plates and back-calculating the density based on the number of dilutions. With regard to the anaerobic NS plates, since no other electron acceptor was available in the medium other than Cr(VI), the CFU on those plates must have respired with Cr(VI) and were therefore denoted as the chromium-reducing bacteria (CRB) population.

#### Most Probable Numbers (MPN).

A 1 g sample of each sediment layer was inoculated in anaerobic growth medium for enumeration of culturable sulfate-reducing bacteria (SRB). SRB liquid growth medium consisted of Postgate's B medium (Postgate, 1984) supplemented with lactate (15 mM) and NaCl (1.5% w/v; designated PB medium). 1 mL of the initial inoculum was sequentially diluted by 10X to a final 10<sup>-9</sup>-fold dilution. Each series was performed in triplicate. Anaerobic dilution tubes were scored for SRB by noting the presence of a black FeS precipitate after 21 days at 26°C. MPN were determined using the program MOST PROBABLE NUMBER CALCULATOR<sup>®</sup> Version 4.04 (Klee, 1996).

#### Microbial Identification.

A set of CFU arising on anaerobic NS plates from water and sediment samples was selected and pure cultures were screened using the BIOLOG<sup>™</sup> Microstation System (BIOLOG, Inc., Hayward, California) according to the manufacturer's instructions. One isolate, designated AR-4, was identified by polymerase chain reaction (PCR) amplification and direct sequencing of the 16S rRNA gene. Isolate AR-4 was grown in Luria-Bertani (LB; Difco Laboratories, Detroit, Michigan) broth overnight, an aliquot (0.5 mL) was centrifuged at 12,000 rpm and the supernatant discarded. The cell pellet was resuspended in 50 µL of PCR Core System I (Promega Corp., Madison, Wisconsin) and 100 pmol each of the forward and reverse primers was added. The universal 16S rRNA primers used were: Primer 375 with the sequence (5'-3'): CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC CCG TCA ATT CCT TTG AGT TT (forward) and Primer 371 with the sequence (5'-3'): CCT ACG GGA GGC AGC AG (reverse). A 'hot start' cycle was initiated (92°C for 5 min), followed by 40 cycles under the following conditions: 92°C for 30 sec (denaturing), 55°C for 30 sec (annealing) and 72°C for 1 min (extension). This was followed by a final extension cycle (72°C for 3 min). The product was held at 4°C and PCR products were visualized on a 1.4 % agarose gel. PCR products were purified using Microcon<sup>™</sup> YM-100 filters (Millipore Corp., Bedford, Massachusetts) according to the manufacturer's instructions. The concentration of PCR product was estimated spectrophotometrically by absorbance at 260 nm.

For DNA sequencing, purified PCR product (75 ng) was amplified using the Big Dye<sup>™</sup> Terminator Kit (PE Applied Biosystems, Foster City, California) and 3.2 pmol of 371 Primer according to the manufacturers' protocol. The reaction conditions were: 96°C for 10 sec, 50°C for 5 sec, and 60°C for 4 min (25 cycles). The reaction mixture was held at 4°C, followed by purification of the PCR product through isopropanol precipitation. Samples were loaded into an ABI Prism 310 Genetic Analyzer (PE Applied Biosystems, Foster City, California). Approximately 450 bp were sequenced and aligned. Obtained sequences were compared to known sequences in the National

Center for Biotechnology Information (NCBI) database (<http://www.ncbi.nlm.nih.gov/BLAST/>; Altschul et al., 1990).

#### Cr(VI) Reduction Assays.

Selected CFU arising on NS plates were grown aerobically in LB broth in 250 mL flasks on a benchtop stirplate. Upon reaching an  $A_{600}$  of 0.5 (approximately  $10^9$  cells  $\text{mL}^{-1}$ ), the culture was purged with  $\text{N}_2$ , treated with chloramphenicol ( $100 \mu\text{g mL}^{-1}$  final concentration) and supplemented with 5 ppm Cr(VI) in the form of  $\text{K}_2\text{CrO}_4$ . Samples were aseptically removed and Cr(VI) concentration estimated via the diphenylcarbazide method (Clesceri et al., 1998). Cr(VI) reduction rates by the Chesapeake Bay bacterial isolates were compared to those obtained from sterile (uninoculated) media controls and a known metal-reducing bacterium, *Shewanella oneidensis*.

A second set of CFU arising on NS plates was grown in NS broth supplemented with 5 ppm Cr(VI) in the form of  $\text{K}_2\text{CrO}_4$ , in glass flasks on a bench-top shaker for 1 week. Samples were aseptically removed each day and the Cr(VI) concentration was estimated by the diphenylcarbazide method as described previously. Cr(VI) reduction rates by Chesapeake Bay bacterial isolates were compared to those obtained from sterile (uninoculated) media controls and *Shewanella oneidensis*.

Cultures of *S. oneidensis* were incubated with sediment from the upper 2 cm of Bear Creek to evaluate any effect that sediment microorganisms might have on Cr(VI) reduction by this organism. Sediment was mixed with LB broth to a concentration of 10% (w/v) and stirred for 1 h prior to the introduction of Cr(VI) or *S. oneidensis*. Due to the presence of observable sulfides in the sediments, a total of 5 ppm of Cr(VI) was added slowly over a period of 2-3 hours to saturate any  $\text{S}^{2-}$  that might potentially reduce Cr(VI), and thus compete with consortia microorganisms or *S. oneidensis*, during experiments. After the saturation period, 10 ppm Cr(VI) and a 2% inoculum of *S. oneidensis* was added to the sediment/LB slurry. Levels of Cr(VI) were monitored as before. Controls consisted of sediment slurries lacking *S. oneidensis* and slurries using autoclaved sediment.

## RESULTS AND DISCUSSION

### Metal Analysis of Bear Creek Sediment.

Concentrations of Cr, Fe, Zn and Ni were determined from sediment using a hot carbonate-hydroxide extraction procedure (James et al., 1995). Recovery of Cr(VI) with this method is greater than 90% based on studies using Cr(VI)-spiked loam soil and sand (James et al., 1995). Experiments in our lab with Cr(VI)-spiked illite clay and organic-rich soil also exhibited recoveries of greater than 90% (unpublished data). Estimates of sediment metal concentration (Table 1) were determined with flame atomic absorption spectroscopy (FAAS). Values for the various metals ranged from a high of greater than 17 ppm to below detection limits. Low level detection limits of FAAS are metal-specific but in general range from 0.001 ppm (Zn) to 0.02 ppm (Fe) (Thompson et al., 1978; Slavin, 1978; Cantle, 1982). With the exception of Ni, all metal concentration estimates for Bear Creek sediments were within detection limits for the method.

Iron is important in the transport of Cr, as Fe(II) can chemically reduce soluble Cr(VI) to insoluble Cr(III), and zinc and nickel are often co-contaminants in chromium-



TABLE 1. Depth concentration profile for total chromium and other metal contaminants in a representative Chesapeake Bay sediment from Bear Creek (Dundalk, Maryland).

Sediment Sample <sup>a</sup>	Parts per million (ppm)			
Depth (cm)	Cr	Fe	Zn	Ni
0-2	0.265	14.485	5.000	0.200
2-4	0.156	17.030	2.886	0.040
4-6	0.385	14.149	2.643	0.000
6-8	0.260	17.812	2.914	0.760
8-10	0.328	2.604	5.200	0.000
10-12	0.338	15.050	3.400	0.120
12-14	0.302	9.307	4.771	0.000
14-16	0.603	8.931	2.157	0.000
16-18	0.270	12.742	2.314	1.080

<sup>a</sup>Sediment cores were sectioned and extracted using a carbonate-hydroxide buffer procedure (James et al., 1995). Aqueous extracts were analyzed by Flame Atomic Absorption Spectroscopy (FAAS) using an air-acetylene flame. Measurements were compared to standard curves of purified standards. Values are the mean of duplicate measurements.

bearing wastewaters (Germain and Patterson, 1974). In addition, studies in our laboratory and others indicate that toxic metals inhibit chromium reduction and growth of chromium-tolerant microorganisms in the laboratory (Lowe et al., 2002; Garbisu et al., 1997; Hardoyo et al., 1991). In Bear Creek sediments, chromium concentrations ranged from 0.156 ppm (2-4 cm depth) to 0.603 ppm (14-16 cm). These levels were significantly lower than previous measurements (Baker et al., 1997). In that report, Cr levels as high as 1,800 ppm were measured. However, they utilized different methods of analysis than those used here and sampled in different locations. The samples in the present study were taken more than 1 km upstream from those in the previous study, were located near-shore rather than in the center of the creek and were collected 4 and 5 years later. Baker et al. (1997) contend that significant spatial differences in contaminant concentrations exist within the Bay. Other investigators agree with this conclusion (Pritchard and Schubal, 2001). Tidal currents, river input, seasons, vertical and horizontal mixing, salt gradients, winds and proximity to contaminant sources all contribute to differential dispersal and accumulation patterns of contaminants in the Bay and thus could account for the large variances in contaminant concentrations from this study to that of Baker et al., 1997. However, the values for Cr(VI) found at the sites sampled are higher than natural chromium levels found in aquatic environments, which typically range from 0.5 to 2 ppb (Shiller and Boyle, 1987).

Iron is often a limiting nutrient in aquatic environments (Sunda, 2000). Concentrations of Fe were highest in the upper regions of the sediment and declined in the lower regions of the sediment column. The highest value for Fe was obtained at a depth of 6-8 cm while the lowest value for Fe corresponded to a depth of 8-10 cm. Concentrations of Zn fluctuated in the sediment and ranged from approximately 2 to 5 ppm. Concentrations of Ni were low or below detection in most of the sediment samples. The highest Ni value (1 ppm) was obtained at the bottom of the sediment core (16-18 cm).

TABLE 2. Depth profiles of aerobic and anaerobic microbial populations in Chesapeake Bay sediments (Bear Creek, Dundalk, Maryland).

Depth (cm)	Total Aerobic (CFU g <sup>-1</sup> ) <sup>a</sup>	Total Anaerobic (CFU g <sup>-1</sup> ) <sup>a</sup>	SRB (Cells g <sup>-1</sup> ) <sup>b</sup>	CRB (CFU g <sup>-1</sup> ) <sup>c</sup>
0-2	3.6 x 10 <sup>5</sup>	2.6 x 10 <sup>4</sup>	1.1 x 10 <sup>5</sup>	6.3 x 10 <sup>4</sup>
2-4	1.9 x 10 <sup>5</sup>	1.7 x 10 <sup>4</sup>	4.2 x 10 <sup>2</sup>	2.6 x 10 <sup>4</sup>
4-6	1.6 x 10 <sup>5</sup>	0.3 x 10 <sup>4</sup>	2.4 x 10 <sup>4</sup>	2.3 x 10 <sup>4</sup>
8-10	0.9 x 10 <sup>5</sup>	0.2 x 10 <sup>4</sup>	9.3 x 10 <sup>2</sup>	0.7 x 10 <sup>4</sup>
14-16	2.8 x 10 <sup>5</sup>	0.8 x 10 <sup>4</sup>	1.9 x 10 <sup>4</sup>	0.4 x 10 <sup>4</sup>
16-18	3.4 x 10 <sup>5</sup>	0.3 x 10 <sup>4</sup>	2.4 x 10 <sup>2</sup>	0.9 x 10 <sup>4</sup>
Average	2.3 x 10 <sup>5</sup>	1.0 x 10 <sup>4</sup>	2.3 x 10 <sup>4</sup>	2.2 x 10 <sup>4</sup>

<sup>a</sup>Total aerobic and anaerobic community estimates were generated from incubations on Nutrient agar (NA) plates supplemented with 1.5% NaCl (w/v).

<sup>b</sup>Sulfate Reducing Bacteria (SRB) were estimated by Most Probable Number (MPN) analysis using PB medium.

<sup>c</sup>Chromium (VI) Reducing Bacteria (CRB) populations were estimated from 21 day anaerobic incubations on NS medium plates.

### Aerobic and Anaerobic Microbial Populations from the Bear Creek Sediment.

After aerobic incubations of the Bear Creek sediment on NA plates, colony counts were obtained (Table 2). Concentrations of aerobically-grown microbial colonies were relatively constant throughout the sediment column with the exception of a section corresponding to a depth of 8-10 cm, in which the concentration of culturable bacteria was 2 to 4 times lower. The highest concentration of anaerobic CFU arising on NA was obtained in the uppermost layer (0-2 cm) of the sediment. The mean concentration of culturable aerobic bacteria for the entire sediment column was  $2.33 \times 10^5$  CFU g<sup>-1</sup> and the microbial community was estimated to be on average 38% Gram-positive and 62% Gram-negative (data not shown). It should be noted that microbial populations are underestimated by cultivation techniques, which probably represent less than 1% of the native microbial community in marine/estuarine environments (Amann et al., 1995). This is due to an inability in the laboratory to duplicate the *in situ* environmental conditions for cultivating microbes (i.e., laboratory media may lack a required nutrient; microscale changes in temperature, pH or O<sub>2</sub> concentration may be significant; microorganisms may have symbiotic or commensal relationships with other biota, etc.)

CFU from anaerobically-grown NA incubations were highest in the upper 4 cm and declined with depth (Table 2). As in the aerobic population, the highest CFU estimate was obtained in the uppermost regions of the sediment column and the lowest value was located at approximately 8-10 cm. The decline in microbial population at 8-10 cm, both aerobically and anaerobically may be due in part to iron limitation (Table 1). The mean of the anaerobically-grown population for the entire length of the sediment core was  $9.6 \times 10^3$  CFU g<sup>-1</sup>, which was 2 orders of magnitude lower than that for the aerobic community.

Many sulfate-reducing bacteria (SRB) are difficult to cultivate on solid media. Therefore, SRB population estimates were made in liquid cultures using Most Probable Number (MPN) analysis. MPN were in the range of 10<sup>2</sup> to 10<sup>5</sup> CFU g<sup>-1</sup> (Table 2). SRB were observed at every depth tested with two spatially-distinct primary populations:

TABLE 3. Population estimates of Chromium (VI) Reducing Bacteria (CRB) from water samples taken from the Chesapeake Bay watershed.

Sample <sup>a</sup>	Cr(VI) Reducing Bacteria (CFU g <sup>-1</sup> wet sediment) <sup>b</sup>
HP	3.5 x 10 <sup>4</sup>
FP	2.5 x 10 <sup>4</sup>
FM	4.4 x 10 <sup>4</sup>
SP	4.3 x 10 <sup>3</sup>
AR	1.5 x 10 <sup>3</sup>

<sup>a</sup>Locations HP, FP and FM were near Baltimore City; samples SP and AR were farther away. See text for detailed descriptions of sampling locations.

<sup>b</sup>Water samples were diluted, spread onto NS media plates and incubated anaerobically for 21 days. CFU, colony forming units.

one located in the upper layer of the sediment and one at a depth of 14-16 cm (Table 2). SRB are potentially important members of the Chesapeake Bay microbial community with respect to chromium contamination. Reduction products of sulfate (SO<sub>4</sub><sup>2-</sup>) respiration by SRB can chemically reduce Cr(VI) (Beukes et al., 1999; Fendorf et al., 2000). In addition, data suggests that certain SRB can directly reduce Cr(VI) (Tebo and Obraztsova, 1998; Tucker et al., 1998; Smith and Gadd, 2000; Michel et al., 2001). CrO<sub>4</sub><sup>2-</sup> ions are structurally similar to SO<sub>4</sub><sup>2-</sup>. Passage of CrO<sub>4</sub><sup>2-</sup> into cells may occur via sulfate transport pathways (Nies et al., 1998).

Populations of Cr Tolerant Bacteria from the Chesapeake Bay Watershed.

Chromium reducing bacteria (CRB) community estimates were highest in the upper 6 cm of Bear Creek sediment and decreased with depth (Table 2). CFU in the upper regions of Bear Creek were comparable to those obtained for water samples from Baltimore City. CRB from the anaerobic incubations of water samples ranged in concentration from 1.5 x 10<sup>3</sup> to 4.4 x 10<sup>4</sup> cell mL<sup>-1</sup>. Locations in Baltimore City, on average, showed CRB counts 15 times higher than those farther away (Table 3). The highest values were obtained from sites FM and HP in Baltimore City.

Identification and Chromium (VI) Reduction Capacity of Cr Tolerant Isolates from the Chesapeake Bay.

To test the Cr(VI) reducing capacity of CRB isolates, cultures of selected CRB were initially grown to a density of approximately 10<sup>9</sup> cells mL<sup>-1</sup> in LB then treated with chloramphenicol to inhibit additional protein production. The media was supplemented with 5 ppm Cr(VI) and sampled every 10 min for Cr(VI) concentration. Most CRB isolated from water samples displayed little or no Cr(VI) reduction ability in short term experiments (data not shown). The best reduction was observed for isolates FP-5 (6 ppb min<sup>-1</sup>), AR-4 (6.5 ppb min<sup>-1</sup>) and SP-4 (16 ppb min<sup>-1</sup>) corresponding to 7%, 9% and 16% reduction of added Cr(VI), respectively (Figure 2A). Previous studies in our lab indicate that the experimental error of the diphenylcarbazine method is between 10 and 15% (data not shown). Therefore, the values obtained for these isolates probably do not represent significant Cr(VI) reduction. Cr(VI) reduction rates for isolates from Sandy Point Park and Anacostia River water were similar to those of isolates from water samples in Baltimore City.

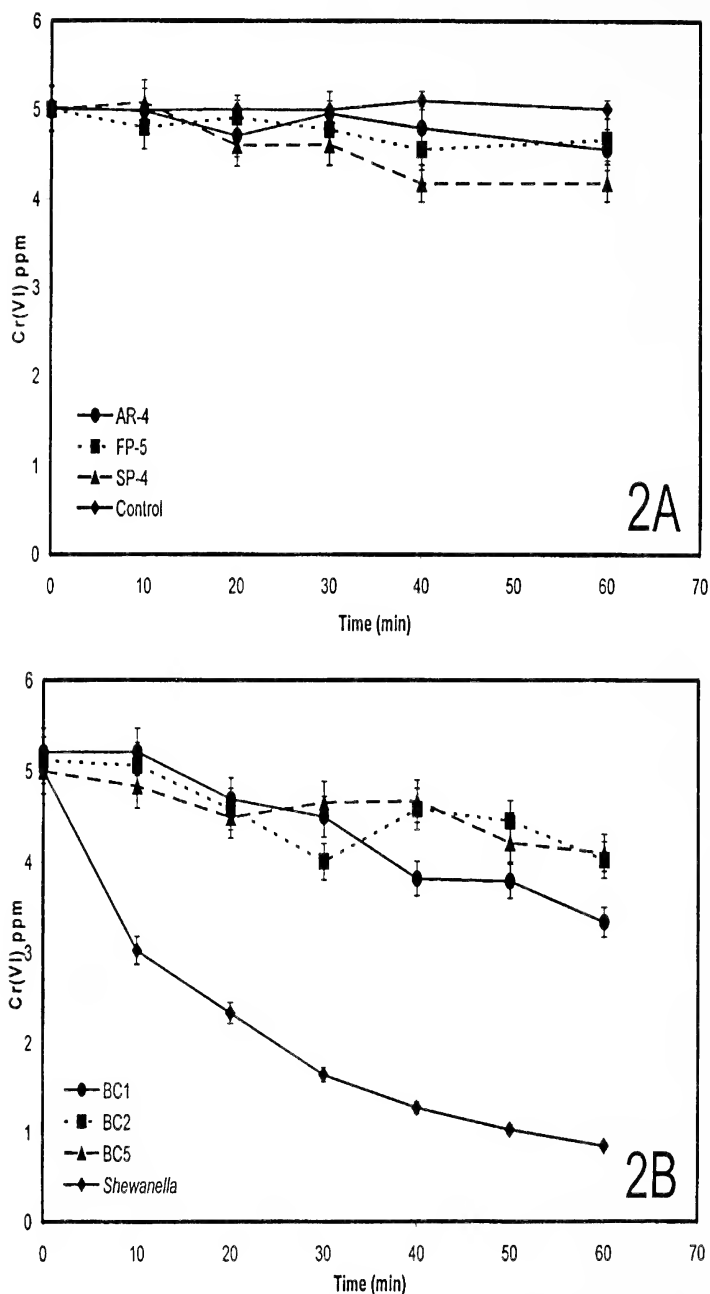


FIGURE 2. Chromium (VI) reduction by isolated Chesapeake Bay chromium reducing bacteria (CRB) and *Shewanella oneidensis*. Cultures were grown aerobically in Luria-Bertani (LB) broth to a density of approximately  $10^9$  cells  $\text{mL}^{-1}$ , treated with chloramphenicol and amended with 5 ppm Cr(VI). Samples were aseptically removed and assayed for Cr(VI) reduction over a 1 h time period. Control cultures consisted of sterile medium. The Cr(VI) concentration was determined using the diphenylcarbazide method (Clesceri et al., 1998). Cr(VI) values are the mean of triplicate experiments with standard error. A) CRB isolates from water; B) sediment CRB isolates and *S. oneidensis*, known metal-reducer

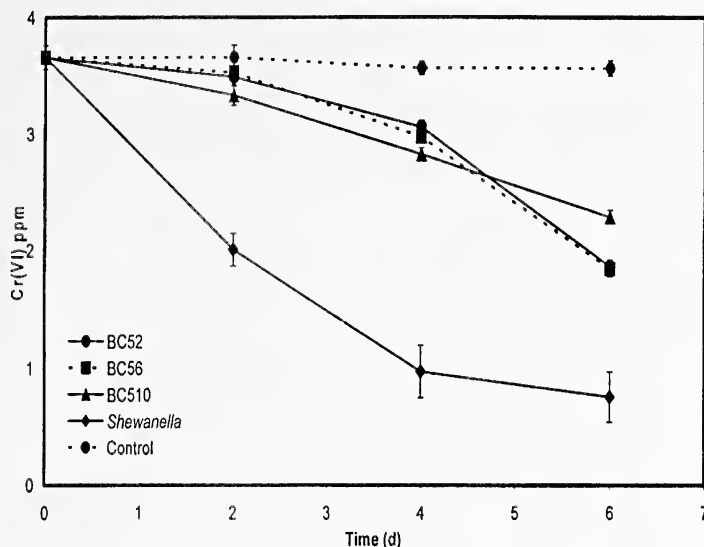


FIGURE 3. Chromium reduction by isolated Chesapeake Bay sediment CRB in a 1-week culture incubation. Isolates were inoculated directly into minimal (NS) medium amended with 3.75 ppm Cr(VI) and incubated aerobically with shaking at room temperature. Samples were aseptically removed and assayed for Cr(VI) reduction. Control cultures consisted of sterile medium. The Cr(VI) concentration was determined using the diphenylcarbazide method (Clesceri et al., 1998). Cr(VI) values are the mean of triplicate experiments with standard error.

Water-borne isolate SP-4 was identified as *Klebsiella pneumoniae* and isolate FP-5 was identified as *Pseudomonas putida* by BIOLOG<sup>TM</sup> analysis. Several other pseudomonads have been reported to reduce Cr(VI). They include *P. ambigua* (Suzuki et al., 1992), *P. stutzeri* (Badar et al., 2000), and *P. synxantha* (McLean et al., 2000). Park et al. (2000) reported the isolation of a chromium (VI) reductase from *P. putida*, however, optimal enzymatic activity for their protein was achieved at pH 5.0 and 80°C. Isolate AR-4 from the Anacostia River was identified as *Kluyvera georgiana* by 16S rRNA sequencing (98% similarity). Members of the genus *Kluyvera* are found in synergistic relationships with terrestrial plants where they are believed to provide the plant with protection from heavy metal toxicity (Burd et al., 1998).

In short-term experiments, Cr(VI) reduction rates by Bear Creek sediment isolates were higher than those for CRB isolates from water samples (Figure 2B). The highest rates were observed in sediment isolates BC-1 (3.5 ppb min<sup>-1</sup>), BC-2 (1.5 ppb min<sup>-1</sup>) and BC-5 (1.5 ppb min<sup>-1</sup>). These were identified by BIOLOG<sup>TM</sup> analysis as being most similar to *Burkholderia* sp., although, the percent similarity to known organisms was less than 50%. Overall, these isolates degraded 36%, 29% and 21% of added Cr(VI) in one hour, respectively. No isolate reduced Cr(VI) at a rate equal to that of *Shewanella oneidensis*, a known metal reducing bacterium (Figure 2B). Rates for *S. oneidensis* were approximately 60 ppb min<sup>-1</sup>, which resulted in an 84% decrease in the Cr(VI) concentration in 1 h. No Cr(VI) reduction was observed in sterile (uninoculated) controls, indicating that Cr(VI) reduction was biologically mediated.

In the Cr(VI) reduction experiments incubated for 1 week, CRB from Bear Creek reduced more Cr(VI) than in short-term experiments (Figure 3). In the short-term experiments, cultures were grown in rich media to a high cell density before the addition of Cr(VI). In the 1 week experiments, cells were inoculated directly into NS medium

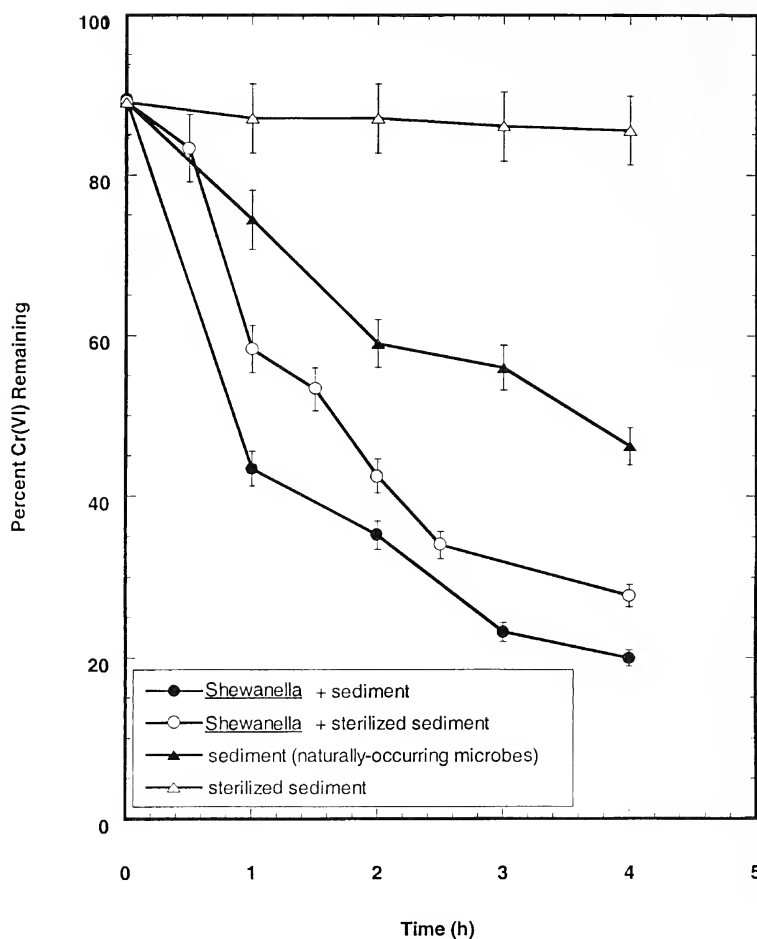


FIGURE 4. Cr(VI) reduction by Bear Creek sediment microbial consortia with and without *S. oneidensis*. Sediment was mixed with LB broth to a concentration of 10% (w/v) and 5 ppm Cr(VI) was initially added to saturate any  $S^{2-}$  that might potentially compete with consortia microorganisms or *S. oneidensis* during Cr(VI) reduction experiments. After  $S^{2-}$  saturation, 10 ppm Cr(VI) and/or a 2% inoculum of *S. oneidensis* was added to the sediment/LB slurry. Levels of Cr(VI) were monitored as before. Controls consisted of sediment slurries lacking *S. oneidensis* and slurries using autoclaved sediment.

supplemented with Cr(VI) and allowed to grow in the presence of the metal. Cultures grew to increasingly high concentrations throughout the first 2 days of incubation, then remained constant at or near concentrations of  $10^4$  cell  $\text{mL}^{-1}$  (data not shown). The highest Cr(VI) reduction by CRB was observed with isolates BC56 ( $0.3 \text{ ppm d}^{-1}$ ), BC52 ( $0.29 \text{ ppm d}^{-1}$ ) and BC510 ( $0.23 \text{ ppm d}^{-1}$ ). These rates were still less than those for *S. oneidensis* ( $0.485 \text{ ppm d}^{-1}$ ). The amount of Cr(VI) reduced in the week-long cultures was typically about 40%, despite the fact that the cultures were one half the concentration of short-term cultures and were growing in a less rich medium.

Slurries of Bear Creek sediment amended with Cr(VI) reduced approximately 48% of the Cr(VI) in 4 h (Figure 4) compared to less than 10% reduction by autoclaved sediment controls. Sterilized sediment inoculated with *S. oneidensis* reduced 69% of the Cr(VI) (Figure 4). When *S. oneidensis* was added to non-sterilized sediment, the

amount of Cr(VI) reduced approached 80%, indicating that *S. oneidensis* enhanced the Cr(VI) reduction by the native consortia (Figure 4).

In conclusion, it would appear that the presence of Cr(VI) is necessary to induce Cr(VI) reduction pathways for some Chesapeake Bay bacteria. By contrast, *S. oneidensis* does not require Cr(VI) induction since *S. oneidensis* cultures rapidly reduce Cr(VI) regardless of prior exposure. We estimated that approximately 80% percent of the Chesapeake Bay microbial community that could be cultured was Cr(VI) tolerant and was found in greater proportion in sediments than in the water column (data not shown). While native Chesapeake Bay microflora are not strong individual candidates for Cr(VI) bioremediation strategies, they do exhibit Cr(VI) tolerance and Cr(VI) reduction. Future work will be necessary to determine the Cr(VI) tolerance strategies (i.e., precipitation at cell surfaces, biosorption, metal-binding protein, etc.) employed by microorganisms in this environment.

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## Identifying Sources of Fecal Pollution in the Roanoke River, Roanoke County, Virginia

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### ABSTRACT

Antibiotic Resistance Patterns (ARPs) of *Enterococcus* spp. were used as a phenotypic fingerprint to compare and categorize unknown-source isolates in an impaired segment of the Roanoke River, Roanoke County, Virginia. Antibiotic resistance analysis (ARA) of enterococci has been effectively used to differentiate among sources of fecal contamination in many geographic regions in the United States. *Enterococcus* spp. were used as a fecal indicator in a library consisting of 1,562 known-source isolates. Two-way analysis indicated that approximately 95% of the unknown-source isolates collected were of animal origin. A 3-way analysis indicated that 61% of the unknowns were of livestock origin while 34% were of wildlife origin. Of the isolates determined to be of wildlife origin, almost all were from raccoons and geese while enterococci from deer were present at low percentages. For one sample date, 20% of the isolates at one site were of human origin. This bacterial source tracking (BST) data will prove valuable for the development of TMDLs for this impaired waterway.

### INTRODUCTION

To date 3,486 km of the 78,000 km of streams and rivers in Virginia are listed as impaired, with only one third being adequately monitored (FORVA). The Roanoke River, used as a source of drinking water and recreation, originates in the mountains of Montgomery County, runs eastward through the highly populated areas of Roanoke County, Salem City, and Roanoke City, continues into North Carolina and empties into Albemarle Sound, North Carolina. In the Roanoke area, land usage is both agricultural, on which horses, cattle and other agriculturally important animals are present, as well as urban from which human indicator bacteria may originate. Large numbers of resident geese and ducks as well as other wildlife are also present.

Of the 803 stream segments in Virginia that are listed as impaired waters, fecal indicator bacteria are the leading cause of the impairment (DEQ). In the next 10 years, Virginia must develop TMDLs (Total Maximum Daily Load) for 600 impaired segments (DEQ). Public watersheds can be restricted from human recreational use if they exceed the Environmental Protection Agency (EPA) standard of 126 *Escherichia coli* or 33 *Enterococcus* colony forming units (cfu) per 100 mL (geometric mean) in fresh water (EPA). Diseases caused by enteric pathogens potentially transmitted through contaminated water include cholera (*Vibrio cholerae*), gastroenteritis (*Escherichia coli*), giardiasis (*Giardia*), salmonellosis and typhoid fever (*Salmonella* sp.), shigellosis (dysentery, *Shigella* sp.), and viruses, such as hepatitis A and Norwalk group viruses (Parveen et al., 1999; United States Environmental Protection Agency, 2001).

With current water testing procedures, the presence of fecal indicator organisms indicate the presence of fecal material but not the source of the contamination. Transforming a non-point source into a point source is valuable in order to improve water quality, reduce the nutrient load leaving the watershed, and prevent possible transmission of disease (Hagedorn et al, 1999). Several methodologies have been implemented to determine human and non-human sources of contamination. While many methods exist, several have been used extensively and successfully, or show promise. These methods include antibiotic resistance analysis (ARA; Wiggins, 1996; Hagedorn et al, 1999; Wiggins et al., 1999; Bowman et al., 2000; Harwood et al., 2000; Bower, 2001), ribotyping (Parveen et al, 1999; Hartel et al., 2000; Carson et al., 2001), pulsed-field gel electrophoresis (PFGE; Simmons, 2000), and utilization of specific carbon sources (Hagedorn, et al., in review).

In the work presented here, Enterococci were used as a fecal indicator for our library of known-source isolates. While fecal coliforms are the standard indicator in Virginia, ARA using the enterococci has been highly successful (Hagedorn et al., 1999; Wiggins et al., 1999; Bower, 2000; Bowman et al., 2000; Harwood et al., 2000). Enterococci are an appropriate indicator in brackish and salt water primarily because they are more apt to survive in marine environments than fecal coliforms because they can tolerate high (6.5%) salt concentrations (Hagedorn et al, 1999). Enterococci also have a higher survival rate through wastewater treatment processes than fecal coliforms making them an attractive target in fresh water (Harwood et al., 2000). This provides the basis for using antibiotic resistance patterns (ARPs) as a "phenotypic fingerprint" to compare and categorize unknown *Enterococcus* spp. isolates. Although antibiotics are primarily used in humans and livestock, we find antibiotic resistance is widespread and common, even in wildlife such as Canada goose, white-tailed deer, muskrat, and raccoon. We report here that ARA of fecal enterococci from known fecal sources, used in conjunction with discriminate analysis (DA), effectively predicted the sources of isolates taken from four Roanoke river sample sites over three sample dates. We have reinforced the bacterial source tracking method and we report both the extent of fecal contamination in the Roanoke River on these dates and the source of fecal contamination.

## MATERIALS AND METHODS

### Bacterial Library

Fresh fecal material, ranging from a swab to several grams, from known sources (horse, human, raccoon, sheep, chicken, cow, white-tailed deer, Canada goose, and muskrat), was diluted in sterile distilled water. Samples from humans are presumed to be a mixture of isolates from several individuals from a portable toilet or pump out truck. Multiple manure samples were collected and mixed, while goose isolates were from single individuals. Several horses were swabbed to obtain fecal material. One hundred L fecal suspension was pipetted onto each mEnterococcus agar (Difco) plate and spread with a sterile glass hockey stick. Plates were inverted and incubated at 37C for 48h. Burgundy and pink colonies were picked off with sterile toothpicks and placed into 200µL enterococcosel broth (BBL) in sterile 96-well plates. Black wells (positive for *Enterococcus* spp.) were noted. A 48 prong replicaplayer was used to transfer isolates onto Trypticase Soy Agar with lecithin and polysorbate 80 (BBL) Antibiotic Plates (Table 1). Antibiotic plates were inverted and incubated for 48h at 37C, and

TABLE 1. Antibiotics and Final Plate Concentration Used for Antibiotic Resistance Analysis.

Antibiotic	Plate Concentration (g/mL)
Chlortetracycline	60,80,100
Oxytetracycline	20,40,60,80,100
Streptomycin	40,60,80,100
Cephalothin	10,15,30,50
Erythromycin	10,15,30,50
Tetracycline	10,15,30,50,100
Neomycin	40,60,80
Vancomycin	2.5
Amoxicillin	2.5

were then evaluated for growth (1) or no-growth (0). Results were entered into the SAS Institute statistical program JMP IN<sup>®</sup> version 4.2. Using discriminant analysis, the library was evaluated for correct classification prior to evaluation of unknown source isolates (Table 2).

#### Bacterial Unknown Source Isolates

Water samples were obtained using standard methods (Greenberg, 1992) from four Roanoke River sample sites spanning approximately 34 kilometers in Roanoke County, Virginia (Roanoke Wayside, 37°14.90N 80°10.48W; Green Hill Park, 37°16.55N 80°06.84W; Route 11, 37°16.10N 80°02.29W; 14<sup>th</sup> Street, 37°15.88N 79°45.94W, obtained via hand-held Magellan GPS 4000). Roanoke Wayside is a small public park adjacent to VA Route 460 upstream from Salem, VA, with a main channel depth at the time of the study of approximately 53cm. Green Hill Park is a large public park just outside Salem City with a main channel site depth of approximately 85cm. The Route 11 site is a shallow site within Salem City with a main channel depth of 26cm. The 14<sup>th</sup> Street site is just upstream from the Roanoke City water treatment facility with a main channel depth of approximately 42cm. Water depths are approximate based on several sample dates from the main channel and could vary seasonally.

Water was filtered on the same day through sterile 0.45µm filters (Gelman), placed onto mEnterococcus agar in 9 X 50mm Petri dishes (Gelman), and incubated for 48h at 37C. The procedure was then identical as for the creation of the library. Once antibiotic growth versus no-growth data was obtained, the isolates were run as unknowns in the library against the known antibiotic resistance patterns (Table 3).

## RESULTS AND DISCUSSION

Antibiotic Resistance Patterns (ARPs) were created for 1,562 enterococci for the development of the library (Table 2). Most published *Enterococcus* libraries range in size from 830 to over 4000 isolates (Harwood et al, 2000; Wiggins et al, 1999; Bower 2000; Graves, 2000; Bowman et al., 2000). The average rate of correct classification (ARCC) of the 2-way analysis of the library was 88% while the ARCC for the 3-way analysis for the library was 81% (Table 2). Three-way wildlife, 4-way livestock and 8-way analyses were also performed (Table 2).

TABLE 2. Rates of Correct Classification (RCC) of Known-Source Library Isolates by Discriminant Analysis

Source	Number of Isolates <sup>A</sup>	Number Correctly Classified	Rate of Correct Classification (%)
2-way			
Animal	1,215	1,154	95
Human	347	277	80
Total	1,562	1,431	88
3-way			
Human	347	264	76
Livestock	804	698	87
Wildlife	411	333	81
Total	1,562	1,295	81
4-way (livestock)			
Chicken	44	21	48
Cow	440	365	83
Horse	235	155	66
Sheep	85	76	89
Total	804	617	72
3-way (wildlife)			
Deer	141	121	86
Goose	174	166	96
Raccoon	94	89	95
Total	409	376	92
8-way			
Horse	235	195	83
Human	347	234	68
Raccoon	94	77	82
Sheep	85	74	87
Chicken	44	36	82
Cow	440	123	29
Deer	141	112	79
Goose	174	127	73
Total	1,560	978	73

<sup>A</sup>Animal, number of samples collected, and average number of isolates per sample:

Horse, 6, 39; Human, 7, 50; Raccoon, 4, 24; Sheep, 2, 43; Chicken, 1, 44; Cow, 6, 62; Deer, 6, 24; Goose, 4, 44; Muskrat, 1, 2. Muskrat isolates excluded from 3-way wildlife library and 8-way library analyses.

An average of 41 unknown-source *Enterococcus* strains were examined per Roanoke River site per sample date. Two-way analysis indicated that approximately 95% of the unknowns collected were of animal origin (Table 3). Our 3-way analysis indicated that approximately 61% of the unknowns were of livestock origin while approximately 34% were of wildlife origin.

TABLE 3. Two-way, Three-way, and Eight-way Classifications of Unknown-Source *Enterococcus* Isolates by Discriminant Analysis

Sample Site/ Date	CFU/ 100mL	Number of Isolates	2-Way Classification%		3-Way Classification %			8-Way <sup>A</sup> Classification%								
			Animal	Human	Human	Livestock	Wildlife	Horse	Human	Raccoon	Sheep	Chicken	Cow	Deer	Goose	
SEPTEMBER																
Roanoke Wayside	364	44	98	2	2	43	55	43	2	43	0	0	0	0	0	11
Green Hill Park	149	48	98	2	2	63	35	60	0	35	2	0	0	0	0	2
Route 11	256	47	100	0	0	60	40	58	0	34	0	0	0	1	0	6
OCTOBER																
Green Hill Park	47	47	100	0	0	47	53	43	0	53	0	0	0	0	0	4
14th Street	65	38	100	0	0	79	21	76	0	8	0	0	0	3	5	8
JANUARY																
Roanoke Wayside	34	37	92	8 <sup>B</sup>	8 <sup>B</sup>	60	32	57	3	11	16	0	0	0	5	8
Green Hill Park	26	44	80	20 <sup>B</sup>	20 <sup>B</sup>	66	14	70	18	5	0	0	0	0	2	5
Route 11	24	35	94	6 <sup>B</sup>	6 <sup>B</sup>	69	26	69	3	8	3	0	0	3	3	9
14th Street	27	27	96	4	4	63	33	56	4	19	4	0	0	4	4	11

<sup>A</sup>An 8-way classification is given as an indication of specific animal source only and should not be interpreted as accurate as the 2-way and 3-way classifications. Muskrat isolates excluded from 8-way classification.

<sup>B</sup>Percent isolates classified as of human origin that fell above the misclassification rate of animal (2-way), or livestock and wildlife (3-way)

TABLE 4. Analysis of Wildlife Unknowns by 3-Way Wildlife Library

Sample Site/ Date	Number Wildlife Isolates <sup>A</sup>	% Total Sample	Classification %		
			Deer	Goose	Raccoon
SEPTEMBER					
Roanoke Wayside	24	55	8	21	71
Green Hill Park	17	35	0	6	94
Route 11	19	40	5	26	68
OCTOBER					
Green Hill Park	25	53	0	8	92
14th Street	8	21	25	25	50
JANUARY					
Roanoke Wayside	12	32	17	33	50
Green Hill Park	8	20	13	38	50
Route 11	8	23	0	25	75
14th Street	9	33	11	33	56

<sup>A</sup>Based on a 3-way analysis of all unknowns. Muskrat isolates excluded from analysis.

We obtained unknowns of human origin on several sample dates, but only in January did the % classification rise above the error rate for the database. Error is assessed as the average percentage of isolates misclassified as human. For example, in the 3-way analysis in which the sets of data were categorized as human, livestock, and wildlife (Table 2), 30 livestock isolates were misclassified as human (3.7%) and 22 of the wildlife isolates were misclassified as human (5.4%) yielding an average incorrect rate of classification (IRCC) of 4.5%. Therefore, any unknown set yielding a number of isolates from human sources above 4.5% would be considered significant. In the January sample at Green Hill Park, 20% of the isolates were of human origin, and 6 to 8% human isolates were obtained at Route 11 and Roanoke Wayside respectively (Table 3).

The work presented here shows the usefulness of ARA in the source tracking of fecal contamination in the Roanoke River. While the results of this study are encouraging, and is essential in the development of TMDLs for this impaired waterway, it again emphasizes the need for multi-year studies to determine the seasonality of sources over time. Virginia is required to develop TMDLs for 600 impaired waters over the next 10 years. Based on current ARA methodology, a 2-way (animal and human) and 3-way analysis (human, livestock, wildlife), or small sub-libraries such as "wildlife" appear to be the most accurate analyses for any known-source database (Hagedorn, personal communication).

Our library had good average rate of correct classification (ARCC), ranging from 72-92% which is good compared to published percentages (54-91%, Wiggins et al., 1999; 73% Human and 89% cattle, Bower, 2000; 87-94%, Graves, 2000; 34-88%, Harwood et al., 2000). The 3-way wildlife library (92% ARCC) and 8-way classifications (73% ARCC) prove to be of interest because of their specificity. One must use caution, however. Unknown isolates indicated as non-human may most effectively be



analyzed in a specific non-human sub-library such as our 3-way wildlife library (deer, goose, raccoon, Table 2) with an ARCC of 92%. In the analysis of unknowns determined to be from wildlife, based on the 3-way wildlife library, most isolates were determined to be from raccoon or goose (Table 4). We feel this approach is better suited to determination of the origin of specific isolate than does an 8-way library (ARCC of 73%) which includes all animals including humans. The creation of sub-libraries (exclusion of human-source isolates for example) controls the number of possible sources and therefore potentially reduces misclassification. This is indicated by the ARCC for each: 92% and 73%, 3-way wildlife and 8-way respectively. Even the 8-way database however indicated that most of the wildlife isolates were from raccoon and goose, and had a respectable ARCC. Over the three sample dates, the source of fecal contamination was generally from the same sources.

An average of 61% of the unknown-source isolates were classified as livestock, with an average of 59% of these over the three sample dates classified as from horse based on the 8-way analysis (Table 3). We know based on general observation that both horses and cattle are present along the river. While the rate of correct classification (RCC) for horse in the 8-way library was 83%, it was only 29% for cow. Cow was misclassified as horse for 32% of the known-cow isolates. We feel therefore that many of the unknowns classified as horse may actually have been from cattle. In the 8-way analysis, only 2% of horse isolates were misclassified as cow, with horse isolates most often misclassified as goose (8%). We feel that with most of the cattle isolates originating in Montgomery County, the regional difference may have contributed to this error rate. While many of the samples originated in both Montgomery (upstream from Salem) and Roanoke Counties as well as Salem City, these specific populations of bacteria may have differed significantly from those actually entering the river. If cattle are suspected contributors of fecal pollution, further specific isolation and comparison would be needed. We feel future studies should also include known-source isolates from urban animals such as cats and dogs, as well as resident ducks. Pigeons may also be an important contributor of fecal material in urban settings such as Salem, as the birds are known to frequent bridges as well as power lines suspended over water.

Using ARPs with discriminant analysis may prove to be a more efficient methodology for bacterial source tracking when compared to molecular methods. Although molecular methods may provide more precise identification of specific types of sources, they have high per-isolate costs, very complex and time-consuming procedures, and are not as feasible in assaying large numbers of samples in a reasonable time frame. We feel that while ARA may not be an appropriate method to discriminate to the level of specific animal using a single multi-animal library, current levels of classification are quite useful. We speculate however that adding a molecular component to ARA may aid in the precision of the analysis. With ARA one may quickly assess general sources of contamination while a molecular methodology may indicate more precisely the specific source if needed. We feel studies are needed comparing multiple methodologies, such as ARA, Ribotyping, PFGE, and Biolog, simultaneously to determine the limitations of each technique.

To date, molecular methods offer similar ARCCs as ARA (Matched 71% of isolates, Samadpour and Chechowitz, 1995; 83% 2-way discriminant analysis, Perveen et al., 1999; 78-100% band matching, Dombeck et al., 2000; 51% band matching, Simmons et al., 2000; 48-96%, Carson et al., 2001). We also recommend samples be

taken from our sample sites during summer months with more frequent sampling, possibly at high and low water events. We reason that greater contamination by human isolates will be evident during higher water events (personal communication Bill Tanger, FORVA) which may have been a contributing factor for the higher human-source *Enterococcus* numbers in January.

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## **Longevity Record For A Wild Allegheny Woodrat (*Neotoma magister*) In West Virginia**

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### **ABSTRACT**

The Allegheny woodrat (*Neotoma magister*) is found throughout much of the central and southern Appalachians and adjacent portions of the Interior Highlands. Allegheny woodrats have declined in the northern portions of their range and are state-listed as threatened, endangered or sensitive species of concern in every state where they occur. Until recently, biologists have had to rely on biological data collected from the closely related eastern woodrat (*N. floridana*) because of limited research on the Allegheny woodrat. We have been studying the ecology and natural history of woodrats in Virginia and West Virginia since 1990. On 8 August 1997 we caught and ear-tagged a juvenile female woodrat. She was caught a total of 24 times in the same outcrop from 1997 through 2002. A conservative estimate of her age on 25 January 2002 was 1,734 days or 57.8 months. This extends the record longevity for a wild Allegheny woodrat by 70 days or 2.3 months. Regardless, her known time alive (from first capture to last) of 1,630 days still surpasses previous estimates of longevity for the Allegheny woodrat

### **INTRODUCTION**

The Allegheny woodrat (*Neotoma magister*) is found throughout much of the central and southern Appalachians and adjacent portions of the Interior Highlands. It is widespread but uncommon in Virginia and West Virginia (Mengak, 1998). It is a habitat specialist closely associated with rock outcrops, cliffs, talus slopes, boulder fields and cave entrances. Allegheny woodrats are tolerant of a wide range of macrohabitats but select specific habitats based on microhabitat features (Castleberry *et al.*, 2002b). The Allegheny woodrats' natural history and role in the local food web and in forest dynamics is unclear. Fungi and mast (hard and soft) are major components of the woodrats diet (Castleberry *et al.*, 2002a) but it is not known what role woodrats play, if any, in the distribution of mycorrhizal fungi and forest regeneration. In this ecoregion, the rough and inaccessible areas inhabited by woodrats generally have not been subject to direct, large-scale disturbances from human activities such as logging, agriculture, or second-home development. The long-term effect of disturbances to

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adjacent habitats on woodrat populations is unclear even as regional land use activities such as forest management and mining continue to increase in intensity. Natural history information on woodrats is needed to assess population status and recommend actions to ensure the long-term survival of this species.

Allegheny woodrats have declined in the northern portions of their range and are state-listed as threatened, endangered or sensitive species of concern in every state where they occur (Beans, 1992; Laerm *et al.*, 2000; Castleberry *et al.*, 2002a). Nonetheless, they still appear to be abundant in appropriate habitat in the central Appalachians of Kentucky, Virginia and West Virginia. Reasons for decline are unclear and are the subject of debate but likely include severe weather (Nawrot and Klimstra, 1976), increased avian and meso-mammal predation (Balcom and Yahner, 1996), reduced hard mast production brought about by the elimination of the American chestnut (*Castanea dentata*) and gypsy moth (*Lymantria dispar*) infestation in oak (*Quercus* spp.) forests (Hall, 1985), vegetation change from white-tailed deer (*Odocoileus virginianus*) herbivory (Hassinger *et al.*, 1996), raccoon roundworm (*Baylisascaris procyonis*) parasitism (McGowan and Hicks, 1996) and habitat fragmentation (Balcom and Yahner, 1996).

Until recently, biologists have had to rely on biological data collected from the closely related eastern woodrat (*N. floridana*) because of limited research on the Allegheny woodrat. However, numerous recent reports have addressed the ecology and natural history of the Allegheny woodrat including studies on landscape characteristics (Balcom and Yahner, 1996), population genetics (Castleberry *et al.*, in review), effects of timber management (Castleberry *et al.*, 2001), summer microhabitat selection (Castleberry *et al.*, 2002b), food habits (Castleberry *et al.*, 2002a), ectoparasites (Castleberry *et al.* in review), reproduction and juvenile growth (Mengak, 2002), and longevity (Mengak, 1997; 2000). With this note, we extend the record for longevity in a wild Allegheny woodrat and comment on reproductive strategy.

Previous records for longevity by a wild Allegheny woodrat were 1,468 days and 1,502 days (Mengak, 1997). An additional record for longevity was reported as 1,664 days (Mengak, 2000). Prior to the work by Mengak (1997; 2000) reported lifespans for Allegheny woodrats were up to 48 months in captivity (Poole, 1940). Other reports for the genus include 991 days for wild eastern woodrats (Fitch and Rainey, 1956), 67 months for captive desert woodrats (*N. lepida*; Landstrom, 1971) and 60 months for captive white-throated woodrats (*N. albigula*; Landstrom, 1971).

A juvenile woodrat (#607) was first caught on 8 August 1997 on Mead-Westvaco Corporation's Wildlife and Ecosystem Research Forest (WERF) in Randolph County, West Virginia (38° 42'N, 80° 3'W). The WERF is a 3360 ha area reserved for the study of industrial forestry impacts on ecosystems and ecological processes in an Appalachian setting (Ford and Rodrigue, 2001). The WERF has been described in detail in previous reports (Castleberry *et al.*, 2001; Castleberry *et al.*, 2002a; Castleberry *et al.*, 2002b). Mengak (1997; 2000) described the capture and marking methods. She was caught in a large rock outcrop in the Rocky Run drainage at an elevation of 920 m. The 2.5-3 ha site's vegetation primarily was a small sawtimber-sized northern hardwood forest with an abundant rhododendron (*Rhododendron maximum*) and greenbrier (*Smilax* spp.) shrub layer. Her weight at initial capture was 150 g. She was caught a total of 24 times in the same outcrop from 1997 through 2002. At her most recent capture on 25 January 2002, she weighed 285 g. Assuming a birth weight of 17 g (Mengak 1997; 2000) and an average weight gain of 1.26 g/d (Mengak, 2002), her estimated birth date was 25 April 1997. Therefore, a conservative estimate of her age on 25 January 2002 was 1,734 days or 57.8 months. This extends the record longevity

for a wild Allegheny woodrat by 70 days or 2.3 months. Regardless, her known time alive (from first capture to last) of 1,630 days still surpasses previous estimates of longevity (Mengak 1997; 2000).

Because we were conducting intensive studies of woodrats in this area, this individual woodrat has contributed a great deal to our general knowledge of the species. She was radio-tagged for three months during the summer of 1998 as part of a telemetry study (Castleberry *et al.*, 2001). An ear biopsy was taken in October 1997 and used in a study of population genetic structure (Castleberry *et al.*, in review). Fecal pellets were collected from her monthly from autumn 1997 to autumn 1998 to assess food habits (Castleberry *et al.*, 2002a). Ecotoparasites were collected from her on multiple occasions (Castleberry *et al.*, in review). She was known to be lactating on 25 May 1999 and thus is presumed to have had offspring.

The Allegheny woodrat is a poorly known faunal component of the central Appalachians. Information on most aspects of woodrat natural history is unclear or controversial (Mengak, 2002). Longevity information is important in understanding long-term trends in capture data and presence/absence surveys. The slow reproductive rate (Mengak, 2002) and long potential lifespan of woodrats suggests that this rodent is functioning more like a K-selected rather than a r-selected species as normally would be attributed to most small mammals in this environment. Accordingly, conservation strategies designed to protect imperiled rodent species may not be applicable for Allegheny woodrats. We suggest that additional research examining Allegheny woodrat population demographics with emphases on reproduction and survivorship are critical for formulating future management plans for this problematic species.

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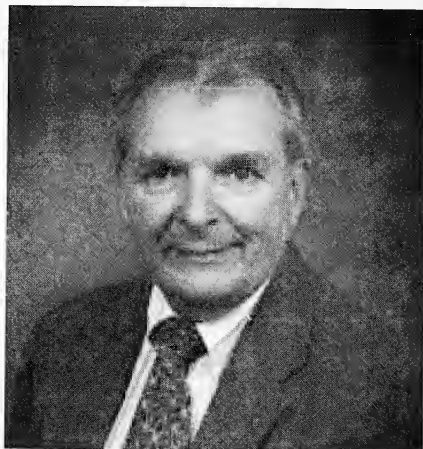
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## **John L. Hess**

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### **Ivey E Lewis Distinguished Service Award**

John L. Hess has been honored as Fellow of the Virginia Academy of Science (VAS) and also awarded the Ivey E Lewis Distinguished Service Award for 2002.

John is not only a well-recognized, active member of the Academy, but also has contributed significantly to science in all three possible categories: as an outstanding scientific researcher, as an inspiring University teacher, and as one who provided significant leadership in the Academy.

John was born in Landisville, Pa., in 1939; he received a Ph.D. degree in biochemistry from Michigan State University in 1966. He has been employed by Virginia Tech since 1967. At present, he is Professor and Head of the Department of Biochemistry at Virginia Tech.

Some of us who have worked with him for VAS and, more recently, on reviewing and editing the manuscript on the history of VAS, realize that John is a very modest person, who would not allow much-deserved praise on his accomplishments or even his photograph to be included in the VAS history. His modesty is probably the reason that his works have not been recognized earlier by the VAS.

His scientific research includes the study of metabolic interactions related to oxidative metabolism and oxidative stress in higher plants and animals. This research has been well-recognized by grants received from federal agencies (USDA and NIH) totaling more than \$419,000 between 1988-1995. His research publications, just between 1990-1997, include 18 co-authored papers. John also was invited to participate in four international meetings between 1993-1998.

The Virginia Tech chapter of the Scientific Research Society of Sigma Xi benefitted from John's work, by selecting him as President-elect, President for 1984-1986, and as Executive Secretary from 1988 to present.

We also recognize in John an excellent college teacher. His classes at Virginia Tech attract the largest number of students that the classroom can hold. For example, during the Spring semester of 1999 he had 110 students in the course: Concepts of Biochemistry. He was awarded the Certificate of Teaching Excellence by Virginia Tech in 1986. He serves as mentor and research advisor for minority college students and to high school students participating in summer programs at Virginia Tech.

John's work for VAS spans the past 26+ years. He served as Director of the Virginia Junior Academy of Science (VJAS) between 1975-1978. Under his leadership, the number of high school students attending and the number of research papers submitted for presentation at the VJAS increased substantially.

During 1995-1997, John co-chaired with Tom O. Sitz the Local Arrangements Committee for the 75th VAS Anniversary Meeting at Virginia Tech in 1997. As it turned out, it was one of the most successful VAS meetings.

John has served VAS with distinction as President during 1999-2000. Most recently, he sacrificed his free summer as a teacher in 2001 to chair the four-member reviewing and editing committee for the publication of the history of VAS. One of the co-signers of this nomination worked closely with John on this project, and can testify that the raw manuscript much benefitted from his work and leadership. To our great relief, the book has just been sent to the printer. This is the reason for not submitting this nomination letter earlier

We all believe that we have an excellent case with John L. Hess, Jr., for recognizing his longstanding distinguished services - as teacher, administrator, and researcher - to the Commonwealth of Virginia and as a volunteer contributor to the Virginia Academy of Science. Therefore we recommend his election to become a Fellow of VAS and also to grant him the Ivey F. Lew/s Distinguished Service Award for 2002.







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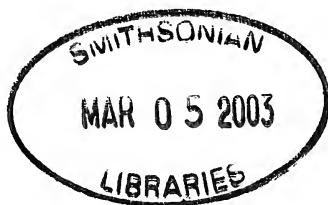
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## A Baseline Assessment of Furbearers on the Upper Coastal Plain of Virginia

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### ABSTRACT

We assessed the status of furbearing mammals on Fort A.P. Hill, Caroline County, Virginia during the 1998-1999 trapping season with the cooperation of local licensed trappers. Our analyses were based on 345 captures representing of 10 mammal species, ranging from one bobcat (*Lynx rufus*) to 157 beavers (*Castor canadensis*). Mean number of captures per 100 trap nights was 17.0. Captures varied from 11.9 to 17.9 per 100 trap nights for conibear traps and 9.7 to 18.3 per 100 trap nights for leg-hold traps. External measurements of six species were similar to those reported for other populations in the region. We suggest that valuable insights into the ecology and trends of furbearer populations can be obtained from studies conducted on government installations such as Fort A. P. Hill. Management plans that include evaluations of infectious disease reservoirs and transmission and impacts of furbearers on wetlands, other wildlife, and human activities would aid in long-term evaluation of these mammals from ecosystem and health perspectives.

Keywords: beaver, *Castor canadensis*, Coastal Plain, furbearer, mammals, morphometrics, raccoon, *Procyon lotor*, trapping, Virginia

### INTRODUCTION

Activities of many mammal species are often erroneously considered detrimental to human progress wherever their habits affects human livelihoods. However, most nuisance mammal species are important components of regional ecosystem maintenance, and when properly managed benefit many facets of human activity. For example, beavers (*Castor canadensis* Kuhl) are directly responsible for creation and maintenance of wetland habitats that support a wide diversity of plants and animals (Naiman *et al.*, 1988, 1994). *Castor canadensis* ponds are used extensively by waterfowl species such as Wood Ducks (*Aix sponsa* L.), American Black Ducks (*Anas rubripes* Brewster), and Mallards (*Anas platyrhynchos* L.) because of suitable microhabitats provided by these wetlands (Merendino *et al.*, 1995). Similarly, many amphibian and reptile species benefit from beaver activity (Mitchell, 1994, 2000). The majority of mammal species addressed in this survey are members of the order Carnivora and many are instrumental in the control of insect pests, rodents, and non-game fish species

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(Godin, 1982; Samuel and Nelson, 1982; Toweill and Tabor, 1982). In addition, many of these species are economically important as furbearers.

The furbearers of the upper Coastal Plain of Virginia have not been studied ecologically (R. Farrar, Virginia Department of Game and Inland Fisheries [VDGIF], pers. comm.). The rich diversity of habitat types found in this region, especially wetlands, should provide suitable habitat for a healthy mammal fauna—including the furbearers (Bellows *et al.*, 2001). The primary objective of our research was to provide a baseline assessment of the mid-sized mammals of a region in Virginia's upper Coastal Plain through analysis of trapping success, demographics, and morphometrics of individuals.

## METHODS

Our research was conducted on Fort A. P. Hill (APH), Caroline County Virginia, located within the Coastal Plain Physiographic Region (centered approximately at 77° 15' N and 38° 05' W). APH is a 30,329 ha field-training military installation in which the majority of the landscape is managed forests and grasslands. Mitchell and Roble (1998) and Bellows *et al.* (1999, 2001) described environmental conditions and habitats on the installation. Bellows *et al.* (2001) provide accounts for all mammal species examined in this paper. Our methods were designed with the cooperation of eight local trappers and the Environmental and Natural Resources Division of APH. Data were collected during the 1998-1999 Virginia trapping season. During this period, trappers provided reports of daily capture success, which included number of each species caught, number of each trap type set, and captures for each trap type. Trap types used fall into two categories, body-gripping (conibear #120, 220, 330) and leg-hold traps (#2 and 3). We recorded species identification, external measurements (total length, tail length, hind foot length, ear length, distance between canines tip-to-tip), demographic data, and body mass for each mammal we processed. Measurements were taken to the nearest mm and mass to the nearest gram.

We calculated total effort, captures per unit effort for each trap type used, and the success of each trap type for each species. Unit of effort = 100 trapnights (TN), and one TN = one trap open for a 24-hr period. We calculated ranges and means ( $\pm 1$  standard deviation) of external measurements and body mass for each sex of each species. For species with  $n \geq 16$ , we used two-sample *t*-tests to compare body mass and body length between males and females ( $\alpha = 0.05$ ; Zar 1996).

We qualitatively assessed habitat characteristics of 13 trapping areas used by four trappers. We recorded dominant tree species, overstory and understory, and shrubs. We also described plant species present at the wetland-upland interface (edge). The presence or absence of four types of evidence of beaver activity (lodge, runways, dams, tree cuttings) also was recorded.

## RESULTS

### Captures

Total reported captures was 345 individuals representing 10 mammal species (Table 1). Number of captures for each species ranged from 157 for *C. canadensis*, representing 46% of reported captures, to one for bobcat (*Lynx rufus* Rafinesque) (Table 1). Raccoons, *Procyon lotor* L., ( $n=65$ , 12%) and muskrats, *Ondatra zibethicus* L., ( $n = 42$ , 19%) were commonly captured. Captures per 100TNs were based on 288 (1,691TNs) of the 345 captures where trap type was specified. Captures per 100TNs by trap type ranged from 18.3 for #2 leg-hold to 9.7 for #3 leg-hold, and mean captures/100TNs for all trap types was 17.0 (Table 1).



TABLE 1. Total captures<sup>1</sup>, total captures where trap type was specified<sup>2</sup>, total trapnights (TNs), and captures/100TNs by trap type for all species.

Order	Species	Conibear Traps			Leg-hold Traps		Totals <sup>2</sup>	Totals <sup>1</sup>
		#330	#220	#120	#2	#3		
Marsupialia								
	<i>Didelphis virginiana</i>	0	0	4	25	0	29	30
Rodentia								
	<i>Castor canadensis</i>	110	10	0	0	3	123	157
	<i>Ondatra zibethicus</i>	21	9	0	2	0	32	42
Carnivora								
	<i>Vulpes vulpes</i>	0	0	0	5	0	5	5
	<i>Urocyon cinereoargenteus</i>	0	0	1	17	0	18	19
	<i>Procyon lotor</i>	0	0	23	33	0	56	65
	<i>Mustela vison</i>	1	2	0	1	0	4	4
	<i>Mephitis mephitis</i>	0	0	0	11	0	11	11
	<i>Lontra canadensis</i>	7	2	0	0	0	9	11
	<i>Lynx rufus</i>	0	0	0	1	0	1	1
	Captures	139	23	28	95	3	288	345
	Trapnights	718	194	230	518	31	1,691	
	Captures/100TNs	17.8	11.9	13.0	18.3	9.7	17.0	

### Habitats

The 13 trapping sites sampled were all riparian habitats surrounded by uplands; deciduous hardwoods dominated most sites. River birch (*Betula nigra* L.) was the only dominant tree species observed that is obligatorily riparian (Reed, 1988). Oaks (*Quercus* L. spp.) were the dominant overstory tree species in 10 of the 13 sites, with white oak (*Q. alba* L.) being the most common. Other common overstory tree species were northern red oak (*Q. rubra* L.), southern red oak (*Q. falcata* Michaux), tulip-tree (*Liriodendron tulipifera* L.), American beech (*Fagus grandifolia* Ehrhart), and red maple (*Acer rubrum* L.). Hickories (*Carya* spp. Nuttall), chestnut oak (*Q. prinus* L.), and river birch were less frequent, but occurred in substantial numbers at one site each. Pines, Virginia pine (*Pinus virginiana* Miller) and loblolly pine (*Pinus taeda* L.), dominated two sites. Common understory tree species, exclusive of sapling overstory trees, were American holly (*Ilex opaca* Aiton), mountain laurel (*Kalmia latifolia* L.), and flowering dogwood (*Cornus florida* L.). Other understory trees frequently observed were sassafras (*Sassafras albidum* Nuttall), ironwood (*Carpinus caroliniana* Walter), and devil's walking stick (*Aralia spinosa* L.). Two shrubs, blueberries (*Vaccinium* L. spp.) and sweet pepperbush (*Clethra alnifolia* L.), were commonly observed at the edge of most sites. Also present were blackberry (*Rubus* L.), mountain laurel, wax myrtle (*Myrica cerifera* L.), common greenbrier (*Smilax rotundifolia* L.), and deciduous tree saplings. The edges of two sites were relatively open with mature deciduous trees. Downed woody debris (DWD) at most sites were of deciduous trees. Evidence of *C. canadensis* activity varied among the 13 sites, with cuttings of deciduous trees being the most obvious.

### Morphometrics

Results of external measurements, demographic information, and body mass were derived from data collected from 111 captures representing six furbearer species (Table 2). The sex of four *C. canadensis*, and two *O. zibethicus* was not recorded, and body mass for an additional 14 *C. canadensis*, 8 females and six males, was not recorded; these 20 individuals are not included in morphometric analyses (Table 2). Analyses were thus based on 91 individuals representing six species. No female mink (*Mustela vison* Schreber) or red fox (*Vulpes vulpes* L.) were captured.

There were no significant differences ( $t=-0.972$ ,  $P=0.832$ ) in body mass or mean total length ( $t=-0.582$ ,  $P=0.718$ ) between male and female *C. canadensis*. There were no significant differences ( $t=-0.803$ ,  $P=0.218$ ) in body mass or mean total length ( $t=1.291$ ,  $P=0.109$ ) between male and female *P. lotor*.

### DISCUSSION

All 10 species represented in this survey are known to occur in this region (Hall 1981; Webster *et al.*, 1985; Whitaker and Hamilton, 1998); however, between 1977 and 1986 fewer than one *L. rufus* per year was reported taken by trappers in Caroline County (Handley, 1991). Four other mid-sized mammal species that should be common in the region, eastern cottontail (*Sylvilagus floridanus* Allen), woodchuck (*Marmota monax* L.), gray squirrel (*Sciurus carolinensis* Gmelin), and long-tailed weasel (*Mustela frenata* Lichtenstein), were not captured and are not addressed here. These species are not usually captured with the types of traps used in this study. However, *S. floridanus*, *M. monax*, and *S. carolinensis* are prevalent on the installation and all have been observed in many habitat types (Bellows *et al.*, 2001). No captures of domestic dogs (*Canis familiaris* L.) or cats (*Felis catus* L.) were reported.

The use of both conibear and leg-hold traps in a variety of sizes ensured that the widest range of mid-sized mammals was represented. The high mean of captures per 100TNs for all trap types demonstrates their collective effectiveness. Carcasses of two species captured, Virginia opossum (*Didelphis virginiana* Kerr) and striped skunk (*Mephitis mephitis* Schreber), were not made available to us by the trappers. Both species appeared frequently in daily capture reports, and the loss of the information associated with these incidental captures represents a loss of valuable data.

Capture location was not available for all captures, making it difficult to compare capture success among trapping areas (habitat types). Comparison of captures/unit effort is a powerful tool for revealing population and habitat preference trends (Chilelli *et al.*, 1996). With such information, fine-scale patterns of habitat use could be established. Without such data, habitat assessments only demonstrate the habitat types most preferred by trappers.

Four species known to live in close association with aquatic habitats (*C. canadensis*, *O. zibethicus*, *L. canadensis*, *M. vison*) were taken primarily with conibears, which are usually set in or under water. Captures of species with more upland affinities (*D. virginiana*, *M. mephitis*, *L. rufus*, *V. vulpes*, *Urocyon cinereoargenteus* Schreber [gray fox]) dominated samples taken by leg-hold traps that are generally set in more upland situations (Darrell Schwartz [trapper], pers. comm.). *Castor canadensis* was the most frequently captured and measured species. This is not surprising for two reasons. First, #330 conibears are the preferred trap for *C. canadensis* (Bateman, 1973) and were the most frequently used trap type. Second, all 13 trapping sites we assessed showed evidence of recent *C. canadensis* activity.

The large number of *C. canadensis* captured during the 1998-1999 trapping period is a reflection of the large population size that exists on APH. *Castor canadensis*

TABLE 2. Summary of means for total length (TL), tail length (TV), hind foot length (HF), ear length, body mass, and canines tip-to-tip of six furbearer species captured on Fort A. P. Hill, Caroline County, Virginia. Range and mean  $\pm$  one standard deviation are provided. No female *V. vulpes* or *M. vison* were captured. External measurements are in millimeters, mass was measured in grams.

Species	Sex	n	TL	TV	HF	Ear	Body mass	Canine
<i>C. canadensis</i>	M	36	653-1151	155-320	130-207	25-36	5,000-25,000	n/a
			903 $\pm$ 124	238 $\pm$ 37	159 $\pm$ 16	31 $\pm$ 3	11,322 $\pm$ 4,264	n/a
	F	22	620-1101	178-295	117-187	24-36	4,400-20,100	n/a
			924 $\pm$ 155	245 $\pm$ 37	160 $\pm$ 21	31 $\pm$ 3	12,545 $\pm$ 5,235	n/a
<i>O. zibethicus</i>	M/F	58	911 $\pm$ 136	241 $\pm$ 37	160 $\pm$ 18	31 $\pm$ 3	11,786 $\pm$ 4,650	n/a
	M	5	552-611	238-257	81-86	8-17	1,100-1,500	n/a
			576 $\pm$ 26	246 $\pm$ 9	82 $\pm$ 3	13 $\pm$ 4	1,263 $\pm$ 180	
	F	4	528-638	227-253	75-85	13-21	1,250-1,600	n/a
<i>V. vulpes</i> *			579 $\pm$ 45	244 $\pm$ 11	82 $\pm$ 4	18 $\pm$ 3	1,470 $\pm$ 130	
	M/F	9	578 $\pm$ 36	245 $\pm$ 10	82 $\pm$ 3	15 $\pm$ 4	1,378 $\pm$ 181	n/a
	M	1	970	360	149	79	4,900	18
<i>P. lotor</i>	M	10	754-890	199-284	104-114	29-64	3,400-6,000	20-25
			819 $\pm$ 38	232 $\pm$ 29	110 $\pm$ 4	50 $\pm$ 11	4,815 $\pm$ 811	23 $\pm$ 2
	F	6	580-938	200-290	98-116	35-58	3,400-6,000	21-24
			758 $\pm$ 144	241 $\pm$ 35	108 $\pm$ 7	48 $\pm$ 9	4,475 $\pm$ 1,015	23 $\pm$ 1
<i>M. vison</i> *	M/F	16	796 $\pm$ 93	236 $\pm$ 30	109 $\pm$ 5	49 $\pm$ 10	4,688 $\pm$ 811	23 $\pm$ 1
	M	1	641	225	68	19	1,800	12
<i>L. canadensis</i>	M	3	999-1,120	335-423	111-126	12-25	5,750-7,700	18-23
			1,080 $\pm$ 67	397 $\pm$ 54	120 $\pm$ 7	20 $\pm$ 7	6,833 $\pm$ 993	21 $\pm$ 3
	F	3	963-1,170	339-425	113-121	14-24	4,600-9,500	21-23
			1,071 $\pm$ 104	376 $\pm$ 44	116 $\pm$ 4	19 $\pm$ 5	6,933 $\pm$ 2,458	22 $\pm$ 1
* Single individual measured	M/F	6	1,075 $\pm$ 79	387 $\pm$ 46	118 $\pm$ 6	19.5 $\pm$ 6	6,883 $\pm$ 1,678	21 $\pm$ 2

\* Single individual measured

presence on APH is evidenced by the high density of active *C. canadensis* ponds on the post (ASB and JCM, pers. obs.). High density of these ponds is likely a direct result of a lack of development on APH. The primary function of APH is to provide the military with a relatively remote and diverse landscape sufficient for conducting a wide variety troop field-training activities. Thus, beaver activity is generally only kept in check when roadways or training facilities are impacted. Collectively, our observations on APH (1991-2001) indicate that in many respects, *Castor canadensis* functions as a keystone species. This is evidenced by the qualitative and obvious impacts of its activities, e.g., tree cutting and damming, on the hydrological and structural attributes of the wetlands and adjacent habitats. Impacted habitats have undergone shifts in habitat suitability for local flora and fauna that have resulted in changes of community composition. Beaver-maintained wetlands often support large populations of amphibians, reptiles, birds (including waterfowl), fish, and aquatic invertebrates. Many plant species rely almost exclusively on conditions generated by beaver activity. Our capture records based on trap type indicate that most of the furbearer species addressed in this study rely, at least in part, on the habitat created and maintained by *C. canadensis*.

All 13 trapping sites where we qualitatively assessed plant species composition and *C. canadensis* activity were wetland habitats. Deciduous trees, especially *Quercus* spp., dominated the canopies and subcanopies at most sites; however, subcanopy tree species composition was more heterogeneous among sites than canopy or shrub species composition. With the exception of impoundments ( $n = 6$ ), sites were *C. canadensis*-maintained habitats ( $n = 7$ ). Two sites had active *C. canadensis* lodges; some had remnants of old lodges. All sites had recent evidence of *C. canadensis* activity—especially deciduous tree cuttings. Cutting activity and low abundance of pines collectively explain why DWD was mostly deciduous at many sites. There was no obvious correlation between plant species composition at the aquatic and terrestrial interfaces (edges) and frequency of *C. canadensis* cuttings or DWD. We expected to see a positive, qualitative, relationship here, as frequency of DWD or tree cuttings increase (i.e., more sunlight), density of shrubs at the edge should also increase—our observations indicated no patterns among these factors. The most plausible explanation for these results is that most sites (8) are maintained, or manicured, as recreational or troop-training areas. Six of the eight sites are impoundments with trails, picnicking and troop-debriefing areas, and boat landings. All 13 sites were suitable habitat for the species trapped therein (Whitaker and Hamilton, 1998; Bellows *et al.*, 2001).

External measurements for five of the six species measured were generally comparable to those in the literature (Handley and Patton, 1947; Paradiso, 1969; Webster *et al.*, 1985; Cothran *et al.*, 1991; Ernst *et al.*, 1997). Measurements for total body length and tail length for the *O. zibethicus* were slightly higher than specified in Willner *et al.* (1980) but comparable to measurements for regional individuals (e.g., Webster *et al.*, 1985). We found no evidence of sexual dimorphism for body mass or for total body length in *C. canadensis* and *P. lotor*.

All of these mammals can be vectors of disease such as rabies, canine distemper, tularemia, and other parasite-borne diseases. Several of these species are well known to become rapidly over-populated with consequences of infectious diseases, parasites, alteration of local biodiversity, and local extinctions (Garrott *et al.*, 1993). These issues occur at broader scales than at local areas (e.g., military base). Several of the species captured frequently occupy edge habitats. These species, including *P. lotor*, *V. vulpes*, *M. mephitis*, and *D. virginiana*, are responsible for much of the mortality of neotropical migrant bird eggs and nestlings that breed in this region (Wilcove *et al.*, 1986; Meffe and Carroll, 1997). The first three are major predators of freshwater turtles and their

eggs (Ernst *et al.*, 1994; Mitchell, 1994) and also consume a wide variety of human-generated products. The term "subsidized predators" is now commonly used for these species, especially *P. lotor* (Mitchell and Klemens, 2000). These predators are also implicated in the decline of the Bobwhite Quail (*Colinus virginianus* L.); however, habitat fragmentation is the primary reason for low population densities for *C. virginianus* in Virginia (M. Fies, VDGIF, pers. comm.).

The geographic area encompassed by APH provides an excellent arena for examining long-term population trends and habitat availability and use over a large breeding area. Population densities of many animal species fluctuate from year-to-year (Gotelli, 2001) and often track prey species density or mast crops. Therefore, the need for long-term data collection to evaluate such trends is evident. Long-term data collection would also provide additional records for less frequently captured species. Also, the limitation of data collection to the trapping season will reveal only winter patterns of the furbearers.

The furbearer fauna of APH is representative of the upper Coastal Plain of the mid-Atlantic region. Military installations like APH are becoming more ecologically valuable because relatively intact landscapes are quickly disappearing from the region. Such public lands or "refuge-like islands" should be assessed for all taxa. Results of these assessments could be used as yardsticks for determining the effects of landscape-level anthropogenic changes such as deforestation for agriculture and urban and suburban sprawl on regional biota.

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**Four Anharmonic Oscillators on a Circle**  
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ABSTRACT

Four identical, uniformly separated particles interconnected by ideal anharmonic springs are constrained to move on a fixed, frictionless circular track. The Lagrangian for the system is written and then transformed by matrix operations suggested by the symmetry of the arrangement of springs and particles. The equations of motion derived from the transformed Lagrangian yield four natural frequencies of motion.

INTRODUCTION

In this paper, we shall consider an idealized mechanical system of four identical particles constrained to move on a fixed, horizontal circular track. Each particle is connected to its two neighbors by identical massless springs whose motions are also confined to the circle of the track. All motions of the particles and springs are taken to proceed without friction so that no energy imparted to the system will be dissipated as heat. The equilibrium positions of the particles are equally spaced on the circle.

In the past, we have exploited the geometries of coupled systems such as that just described to separate their equations of motion either completely or to a significant extent. After the separated equations of motion were written in terms of symmetry coordinates, it was then not a difficult matter to obtain the natural frequencies of vibration corresponding to the various symmetry coordinates (Boyd and Raychowdhury, 2001c; Boyd, Hudepohl, and Raychowdhury, 2001a; Boyd, Hudepohl, and Raychowdhury, 2001b). In each case, the coupling between neighboring particles was provided by harmonic springs.

More recently, we have used matrix and Lagrangian techniques to discover natural frequencies for the transverse vibrations of a linear array of three Hooke's Law springs and two masses with the two endpoints of the array fixed in space. The transverse vibrations are anharmonic with restoring forces on the masses proportional to the cubes of their displacements away from equilibrium (Boyd, Hudepohl, and Raychowdhury, 2002b).

It has been our ambition for quite some time to apply the techniques which have been successful for coupled harmonic oscillators to systems of coupled anharmonic oscillators. The linearity of the harmonic equations of motion accounts for the relative ease with which we have been able to separate those equations. The nonlinearity of the equations of the anharmonic oscillators challenge the essentially linear techniques which we have been using. This paper represents our first attack upon a fairly complicated anharmonic system.

Harmonic springs provide tensions which are proportional to the amount by which they are stretched or compressed away from their natural lengths. Thus the equations

of motion for harmonic systems are linear. The elastic potential energy of an harmonic spring is proportional to the square of the change in its length by either stretch or compression.

The coupling in the system under study is provided by anharmonic springs. The tensions in such springs are proportional to the cubes of their changes in length, and the elastic potential energy stored in each of these springs is proportional to the fourth power of its change in length.

We have been able to combine the matrix and Lagrangian techniques which were successful for the simple system of two coupled transverse anharmonic oscillators with a transformation suggested by the symmetry operations used in the investigation of the larger systems of harmonically coupled oscillators. The result is that we have obtained natural frequencies of vibration for the four particles on a circle as first described in the case that the springs are anharmonic. We shall describe that work in this paper. Our emphasis will be upon the use rather than the development of the transformation matrices which simplify our computations. The group representation theory underlying the construction of transformation matrices can be found in numerous places (Duffey, 1973; Hammermesh, 1962; Nussbaum, 1968). It was the proper formulation of the potential energy matrices that enabled us to complete our calculations.

#### LAGRANGIAN FOR FOUR PARTICLES COUPLED WITH ANHARMONIC SPRINGS

We represent in Figure 1, the system of four particles and springs constrained to move on their fixed circle. Thus the vibrations of the system will be longitudinal. We denote the mass of each particle by  $m$  and an anharmonic force constant for each spring by  $\beta$ . The spring constant will be defined by the way in which we write the elastic potential energies for the springs. The counter-clockwise displacement of the particles from their equilibrium positions are denoted by  $x_1, x_2, x_3$ , and  $x_4$ . The corresponding velocities of the particles are denoted by  $\dot{x}_1, \dot{x}_2, \dot{x}_3$ , and  $\dot{x}_4$  and the total kinetic energy of the vibrating masses as they move is

$$KE = \left(\frac{m}{2}\right)(\dot{x}_1^2 + \dot{x}_2^2 + \dot{x}_3^2 + \dot{x}_4^2).$$

This total kinetic energy may be written in matrix notation as

$$KE = \left(\frac{m}{2}\right)\dot{X}IX^T$$

where  $I$  represents the 4-by-4 identity matrix,  $\dot{X} = (\dot{x}_1 \dot{x}_2 \dot{x}_3 \dot{x}_4)$  represents the row velocity matrix, and  $\dot{X}^T$  represents the transpose of the row velocity matrix.

The anharmonic springs provide forces proportional to  $|x_j - x_l|^3$  on the  $j$ -th and  $l$ -th particles to which they are attached. These forces tend to restore the particles to their equilibrium positions. We take the elastic potential energy stored in the spring connecting the  $j$ -th and  $l$ -th particles to be  $(\beta/4)(x_j - x_l)^4$  where  $\beta$  is a positive number. The total elastic potential energy for the system becomes

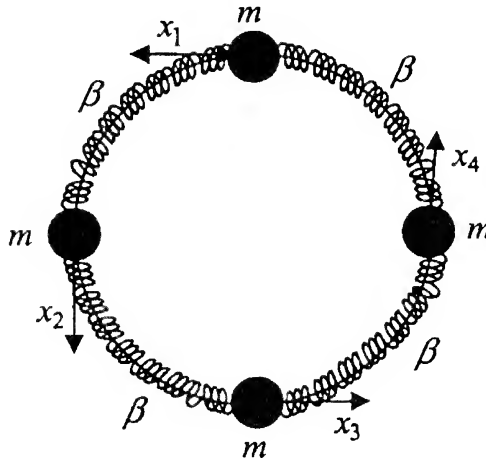


FIGURE 1. The Four Particles on Their Circle.

$$PE = \left( \frac{\beta}{4} \right) \left[ (x_1 - x_2)^4 + (x_2 - x_3)^4 + (x_3 - x_4)^4 + (x_4 - x_1)^4 \right]$$

The potential energy involves the raising of four binomials to the fourth power. Our task is to discover a matrix formulation for the total elastic potential energy of the system which will accomplish the binomial algebra. To continue the notation adopted for expressing the kinetic energy, we let  $X = (x_1 x_2 x_3 x_4)$  represent the row displacement matrix and  $X^T$  the transpose of  $X$ . We then choose the following four potential energy matrices:

$$V_{12} = \begin{pmatrix} 1 & -1 & 0 & 0 \\ -1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}, \quad V_{23} = \begin{pmatrix} 0 & 0 & 0 & 0 \\ 0 & 1 & -1 & 0 \\ 0 & -1 & 1 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix},$$

$$V_{34} = \begin{pmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 \\ 0 & 0 & -1 & 1 \end{pmatrix}, \text{ and } V_{41} = \begin{pmatrix} 1 & 0 & 0 & -1 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ -1 & 0 & 0 & 1 \end{pmatrix}.$$

Straightforward computation will justify the statement that

$$PE = \left(\frac{\beta}{4}\right) \left[ \left(XV_{12}X^T\right)^2 + \left(XV_{23}X^T\right)^2 + \left(XV_{34}X^T\right)^2 + \left(XV_{41}X^T\right)^2 \right]$$

The Lagrangian function for the system of particles and springs is given by  $L = KE - PE$  and we may write that

$$L = \left(\frac{m}{2}\right) \dot{X}I\dot{X}^T - \left(\frac{\beta}{4}\right) \left[ \left(XV_{12}X^T\right)^2 + \left(XV_{23}X^T\right)^2 + \left(XV_{34}X^T\right)^2 + \left(XV_{41}X^T\right)^2 \right]. \quad (1)$$

### A TRANSFORMATION OF THE LAGRANGIAN

We seek to simplify the Lagrangian of our system by means of a transformation based upon the geometry of the circular arrangement of springs and masses. The rigid geometrical symmetries of the springs and masses on their circle are four reflections and counterclockwise, plane rotations of  $90^\circ$ ,  $180^\circ$ ,  $270^\circ$ , and  $360^\circ$  about the center of the circle. Taken together, these eight symmetry operations comprise the nonabelian group  $C_{4v}$  in which the rotation through  $360^\circ$  serves as the identity element. Familiarity with the matrix group representations of  $C_{4v}$  suggested to us that the orthogonal matrix

$$S = \begin{pmatrix} 1 & 1 & 1 & 1 \\ 1 & -1 & 1 & -1 \\ 1 & -1 & -1 & 1 \\ 1 & 1 & -1 & -1 \end{pmatrix} / 2$$

might provide us with a transformation which would simplify the Lagrangian of equation 1 and, hence, the equations of motion which follow from the Lagrangian. The orthogonal transformation with  $S$  together with the choice of the potential energy matrices  $V_{12}, V_{23}, V_{34}$ , and  $V_{41}$  does lead to our goal of simplifying the equations of motion of the system.

Let us transform the coordinate and velocity vectors by

$$SX = S(x_1x_2x_3x_4) = (z_1z_2z_3z_4) = Z, x^T S^{-1} = Z^T,$$

$$S\dot{X} = S(\dot{x}_1\dot{x}_2\dot{x}_3\dot{x}_4) = (\dot{z}_1\dot{z}_2\dot{z}_3\dot{z}_4) = \dot{Z},$$

$$\text{and } \dot{X}^T S^{-1} = \dot{Z}^T.$$

We shall refer to the coordinates  $z_j$ ,  $j = 1, 2, 3, 4$ , as symmetry coordinates. Their corresponding velocities are  $\dot{z}_j$ .

We must also transform the potential energy matrices in a manner consistent with the transformations of coordinates and velocities. Those transformations may be accomplished by the following computations:

$$SV_{12}S^{-1}, SV_{23}S^{-1}, SV_{34}S^{-1}, \text{ and } SV_{41}S^{-1}.$$

The Lagrangian as given by equation 1 may now be rewritten in terms of the symmetry coordinates and their velocities as

$$\begin{aligned}
 L &= \left(\frac{m}{2}\right) \dot{X} S^{-1} S I S^{-1} S \dot{X}^T \\
 &- \left(\frac{\beta}{4}\right) \left( X S^{-1} S V_{12} S^{-1} S X^T \right)^2 + \left( X S^{-1} S V_{23} S^{-1} S X^T \right)^2 \\
 &+ \left( X S^{-1} S V_{34} S^{-1} S X^T \right)^2 + \left( X S^{-1} S V_{41} S^{-1} S X^T \right)^2 \\
 &= \left(\frac{m}{2}\right) \left( \dot{z}_1^2 + \dot{z}_2^2 + \dot{z}_3^2 + \dot{z}_4^2 \right) - \beta \left( z_2^4 + 3z_2^2 z_3^2 + \frac{z_3^4}{2} + 3z_2^2 z_4^2 + \frac{z_4^4}{2} \right).
 \end{aligned} \tag{2}$$

We note that we have resorted to the computer algebra system *Mathematica* to perform the matrix manipulations leading to this expression for  $L$  in terms of  $z_j$  and  $\dot{z}_j$ .

### EQUATIONS OF MOTION AND NATURAL FREQUENCIES

Equations of motion in terms of the new symmetry coordinates and their accelerations are given by

$$\frac{d}{dt} \left( \frac{\partial L}{\partial \dot{z}_j} \right) - \left( \frac{\partial L}{\partial z_j} \right) = 0 \tag{3}$$

Thus, we may write

$$m \ddot{z}_1 = 0, \tag{3.1}$$

$$m \ddot{z}_2 + \beta (4z_2^3 + 6z_2 z_3^2 + 6z_2 z_4^2) = 0, \tag{3.2}$$

$$m \ddot{z}_3 + \beta (6z_2^2 z_3 + 2z_3^3) = 0, \text{ and } \tag{3.3}$$

$$m \ddot{z}_4 + \beta (6z_2^2 z_4 + 2z_4^3) = 0. \tag{3.4}$$

Although equations 3.1, 3.2, 3.3, and 3.4 are not completely separated, we observe that, if we set as initial conditions that all  $z_j = 0$ , and  $\dot{z}_j = 0$  at  $t = 0$  except for  $j = k$ , the equation governing the variation in time of  $z_k$  involves no other symmetry coordinate. Thus the vibrations associated with each of  $z_1, z_2$ , and  $z_4$  may be stimulated and sustained while the other symmetry coordinates remain suppressed as time progresses.

Such would not be the case if we had expanded the original Lagrangian of equation 2 in the coordinates  $x_1, x_2, x_3, x_4$  and velocities  $\dot{x}_1, \dot{x}_2, \dot{x}_3, \dot{x}_4$ . In that

expansion, the appearance of terms of the form  $4x_j x_l^3$  with  $j \neq l$  contribute a coupling in the equations of motion which make it impossible for one coordinate to change without affecting other coordinates.

Returning to equation 3.2, we choose initial conditions  $z_3 = z_4 = 0$  and  $\dot{z}_3 = \dot{z}_4 = 0$ . For equation 3.3, we take  $z_2 = 0$  and  $\dot{z}_2 = 0$ . For equation 3.4, we take  $z_2 = 0$  and  $\dot{z}_2$  as well. Thus we are led to the next four equations of motion from which we can compute the frequencies of the system vibrating in natural or symmetrical modes:

$$m\ddot{z}_1 = 0 \quad (4.1)$$

$$m\ddot{z}_2 + 4\beta z_2^3 = 0 \quad (4.2)$$

$$m\ddot{z}_3 + 2\beta z_3^3 = 0, \text{ and} \quad (4.3)$$

$$m\ddot{z}_4 + 2\beta z_4^3 = 0. \quad (4.4)$$

The symmetry coordinate  $z_1$  corresponds to a rotation at constant angular velocity with frequency  $f_1 = 0$  since there is no vibration. Solutions in closed form for  $z_2, z_3$ , and  $z_4$  may be written with the Jacobi cosine and sine amplitude functions (Dixon, 1984). These functions are denoted by  $cn(t, \alpha)$  and  $sn(t, \alpha)$ , respectively.

They are doubly periodic, analytic functions of the complex variable  $t$ . The parameter  $\alpha$  is known as the modulus of its function. When the independent variable  $t$  is taken to be real valued (as is time in our problem) the functions have only a single period and resemble the trigonometric functions. These functions appear in the exact solutions of the equations of motion for the simple pendulum with large displacements and for a uniform sphere of specific gravity 0.5 bobbing in water (Boyd, 1991). Those readers who wish to investigate those elliptic functions will be interested to know that the functions have been written into the *Mathematica* software (Wolfram, 1999).

We shall simply discover the periods of vibration corresponding to equations 4 by integration. Let us turn to equation 4.3 and suppose that at  $t = 0$ ,  $z_3 = A_3 > 0$  and  $\dot{z}_3 = 0$ . Equation 4.3 may be rewritten as

$$\frac{d\dot{z}_3}{dt} = \dot{z}_3 \frac{d\dot{z}_3}{dz_3} = -\frac{2\beta}{m} z_3^3.$$

The first integration

$$\int_0^{\dot{z}_3} u du = -\frac{2\beta}{m} \int_{A_3}^{z_3} v^3 dv$$

yields

$$\frac{\dot{z}_3^2}{2} = -\frac{2\beta}{m} \left( \frac{\dot{z}_3^4}{4} - \frac{A_3^4}{4} \right) = \frac{\beta}{2m} (A_3^4 - z_3^4).$$

It follows that

$$\frac{dz_3}{dt} = \sqrt{\frac{\beta}{m}} \sqrt{A_3^4 - z_3^4}$$

or

$$dt = -\frac{dz_3}{\sqrt{\frac{\beta}{m}} \sqrt{A_3^4 - z_3^4}}$$

where the negative square root is taken since, as time increases during the first quarter period of motion after  $t = 0$ ,  $z_3$  decreases from  $A_3$  to 0.

Let us denote the period of vibration for symmetry coordinate  $z_3$  by  $T_3$ .

Integrating the last equation from 0 to  $\frac{T_3}{4}$  on the left-hand side and from  $A_3$  to 0 on the right-hand side yields

$$T_3 = 4 \int_0^{A_3} \frac{dz_3}{\sqrt{\frac{\beta}{m}} \sqrt{A_3^4 - z_3^4}} = 4 \sqrt{\frac{m}{\beta}} \int_0^1 \frac{du}{A_3 \sqrt{1 - u^4}} = \frac{1}{A_3} \sqrt{\frac{m}{\beta}} (5.24412).$$

Thus the frequency  $f_3 = \frac{1}{T_3}$  depends upon the amplitude  $A_3$  of the motion as is

known to be the case for the elliptic functions. The integral is an elliptic integral which has been evaluated by *Mathematica*. Inspection of equation 4.4 indicates that

$$T_4 = 4 \sqrt{\frac{m}{\beta}} \int_0^1 \frac{du}{A_4 \sqrt{1 - u^4}} = \frac{1}{A_4} \sqrt{\frac{m}{\beta}} (5.24412)$$

where  $A_4$  is the amplitude of the variation in  $z_4$ . Then  $f_4 = \frac{1}{T_4}$ .

A similar pair of integrations that begin with equation 4.2 leads to the conclusion that

$$T_2 = 4\sqrt{\frac{m}{2\beta}} \int_0^1 \frac{du}{A_2 \sqrt{1-u^4}} = \sqrt{\frac{m}{2\beta}} (5.24412)$$

$$\text{and } f_2 = \frac{1}{T_2}.$$

### CONCLUSION

Since the elliptic functions govern the motions of the system of anharmonic oscillators, the natural frequencies will always depend on the amplitudes of the corresponding vibrations. As previously noted, the Jacobi elliptic functions can be handled in closed form with *Mathematica*. In addition, *Mathematica* permits us to experiment with various matrix forms to develop useful transformations of coordinates. We have taken advantage of this computational power to give exact solutions of equations of motion for a simpler, anharmonic system than that considered in this work (Boyd, Hudepohl, and Raychowdhury 2002a).

We hope to look at other systems, but so far each problem that we have considered has required a solution tailored to the particular problem. It seems clear that no computational program for natural anharmonic frequencies will ever match in elegance and simplicity the symmetry-based calculations for natural harmonic frequencies.

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# **Environmental Factors Contributing to the Disaggregation of a Colonial Cyanoprokaryote and Its Influence on Picoplankton Abundance within Lake Joyce, Virginia**

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## **ABSTRACT**

A colonial cyanoprokaryote, *Aphanocapsa holsatica* and autotrophic picoplankton abundance were monitored weekly over a two year period in Lake Joyce, Virginia. Significant differences were observed in both the cyanoprokaryote and picoplankton abundance over the study period and an inverse relationship was observed between these two plankton groups. Disaggregation of colonies was shown to contribute to picoplankton populations where water temperature and precipitation input apparently trigger colony dispersion. This relationship is suggested to occur in other aquatic habitats. Results of this work and its implications for ecosystem dynamics are discussed.

## **INTRODUCTION**

Picoplankton is defined as plankton between 0.2 and 2.0 $\mu$ m in size (Sieburth et al., 1978) and may include a variety of both heterotrophic and autotrophic organisms (Marshall, 2002). Numerous studies have shown picoplankton as an abundant and productive component within a variety of oceanic, estuarine and freshwater environments (Li, et al., 1983; Fahnensteil and Carrick, 1992; Marshall and Nesius, 1993; Affronti and Marshall, 1994). However, questions remain as to the relationship of picoplankton in aquatic food web dynamics (Stockner and Shortreed, 1989; Fogg, 1995; Marshall, 2002). To answer these questions, more detailed information is required on factors which influence picoplankton composition dynamics. With this information, a better understanding of the availability of picoplankton as a link or sink for nutrients can be determined.

The objectives of this study are: 1) identify variation in both autotrophic picoplankton and colonial cyanoprokaryotic abundance using a high frequency sampling regime and 2) identify the effects, if any that water temperature and storm water runoff have on colonial cyanoprokaryotic and autotrophic picoplankton population dynamics in Lake Joyce, Virginia.

Lake Joyce, Virginia (36° 54' 44'' Lat., 76° 7' 19'' Long.) is a 60ha freshwater lake whose overflow empties via Pleasure House Creek and the Lynnhaven River into the lower Chesapeake Bay. The Virginia Department of Environmental Quality (1994) has described this water body as an unstratified, hypereutrophic system whose average depth is 1.1m. The major nonpoint source input is from urban stormwater runoff. Lake Joyce is representative of other lakes in the Norfolk/Virginia Beach area where its general usage includes fishing, boating, and water skiing.

## **METHODS**

During this study, three replicate surface grab samples (125 mL) were collected weekly at one station in Lake Joyce over a 24 month period (May 29, 2000 to May 20,

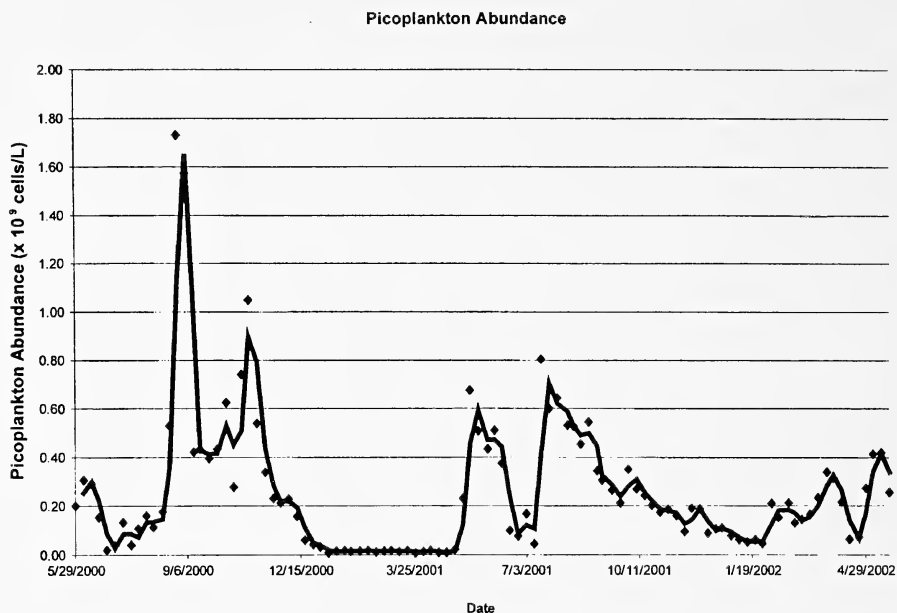


FIGURE 1. Average picoplankton abundance from three replicate samples. A moving average best fit line was used to fit data series.

2002) and preserved in 2% glutaraldehyde. Subsamples (5 mL) were drawn on to a 0.2 $\mu$ m Nucleopore filter stained in Irgalan Black using a mechanical pump at pressures less than 10cm of Hg to prevent cell rupture and colony disturbance. Both picoplankton and colonial cyanoprokaryotic abundance were determined from these filters using a Zeiss Epifluorescence Axiolab Microscope equipped with a 50 watt mercury bulb and a Zeiss 546 excitation filter, FT580 dichromatic mirror and 590 barrier filter. Picoplankton abundance was calculated from three replicate samples and colonial cyanoprokaryote abundance was calculated from two replicate samples. An ocular grid system (10 x 10 square grid, each square measured 10 $\mu$ m squared at 1000X) was used as a template to aid in the counting of both picoplankton and the colonial cyanoprokaryotic organism.

A one way Model I ANOVA with time as a treatment was performed on both picoplankton and colonial cyanoprokaryotic data to determine if significant changes in abundance occurred over the study period. Average picoplankton and colonial cyanoprokaryotic abundance was compared to both water temperature (October 23, 2000 to May 20, 2002) and weekly precipitation data (May 23, 2000 to April 1, 2002) (NOAA, 2000 – 2002).

## RESULTS

The colonial cyanoprokaryote was tentatively identified as *Aphanocapsa holsatica*, according to Komarek (2000). Average picoplankton abundance data for the study period are given in Figure 1. Cell abundance ranged from  $4.74 \times 10^6$  to  $2.23 \times 10^9$  cells/L with peaks observed from late summer to early fall. Results of the one way Model I ANOVA indicated picoplankton abundance was affected by time ( $P < 0.00001$ ). Abundance data for May 29, 2000 was not included in this analysis

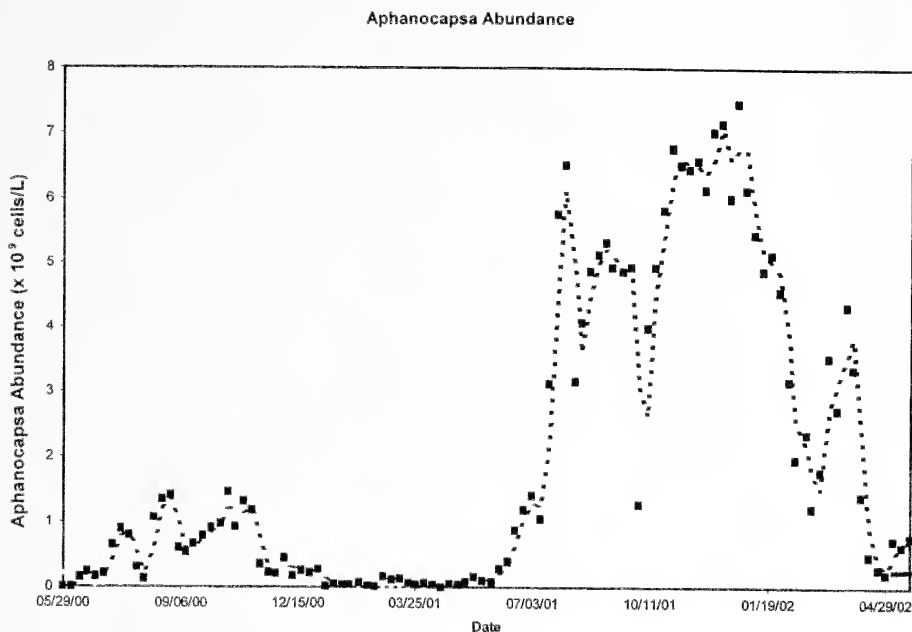


FIGURE 2. Average *Aphanocapsa holsatica* abundance from two replicate samples. A moving average best fit line was used to fit data series.

(missing replicate sample). Results of Tukey's a posteriori tests verified an overall decline in picoplankton abundance over the study period as picoplankton abundance on August 28, 2000 (peak value for 2000) was significantly higher than the maximum abundance observed during the following summer (July 16, 2001).

Average *Aphanocapsa holsatica* abundance data are given in Figure 2. Abundance peaks occurred in summer with a significant and extended growth pattern observed over the second year of the study. *Aphanocapsa holsatica* abundance ranged from 0 to  $8.22 \times 10^9$  cells/L over this two year study. Results of the one way Model I ANOVA indicated *Aphanocapsa holsatica* cell abundance was affected over time ( $P < 0.00001$ ). Tukey's multiple comparison tests suggests an overall increase in *Aphanocapsa holsatica* abundance over the study period. However, its maximum abundance on October 16, 2000 was significantly lower compared to the maximum the following year (December 23, 2001). In comparison, there was an inverse relationship between *Aphanocapsa holsatica* and picoplankton average abundance (Figure 3).

Water temperature taken over 19 months of the study is shown in Figure 4. Water temperature varied from  $0.56^\circ\text{C}$  ( $33.0^\circ\text{F}$ ) to  $30.0^\circ\text{C}$  ( $86.0^\circ\text{F}$ ). Average picoplankton abundance is in phase with water temperature (Figure 4) while average *Aphanocapsa holsatica* abundance is not (Figure 5). Weekly precipitation is shown in Figure 6. Precipitation ranged from 0cm to 11.20cm (4.41in) over the study period. Total rainfall for the first year of the study (115.77cm, 45.58in) was higher than the second year total (66.98cm, 26.37in). Average picoplankton abundance followed precipitation patterns with a slight lag time between their peaks (Figure 6). Average *Aphanocapsa holsatica* abundance had an inverse relationship with the amount of precipitation (Figure 7).

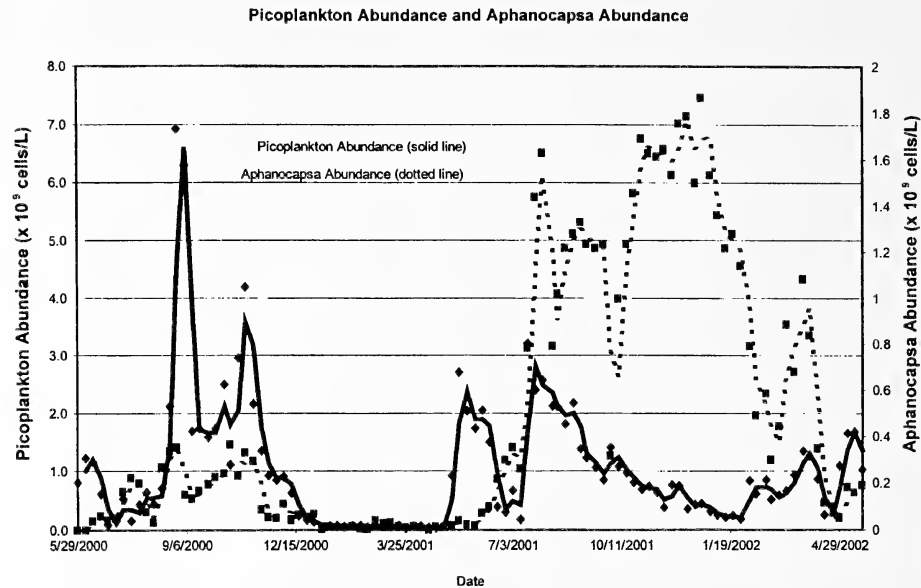


FIGURE 3. Comparison of picoplankton and *Aphanocapsa holsatica* abundance. Best fit lines are moving averages of each data series.

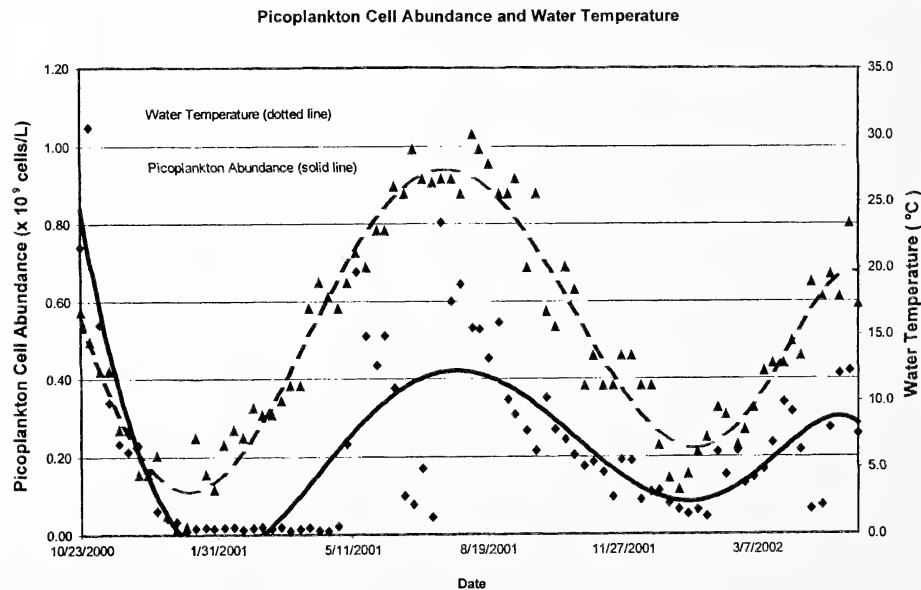


FIGURE 4. Comparison of picoplankton abundance and water temperature. Polynomial best fit lines are used for each data series.

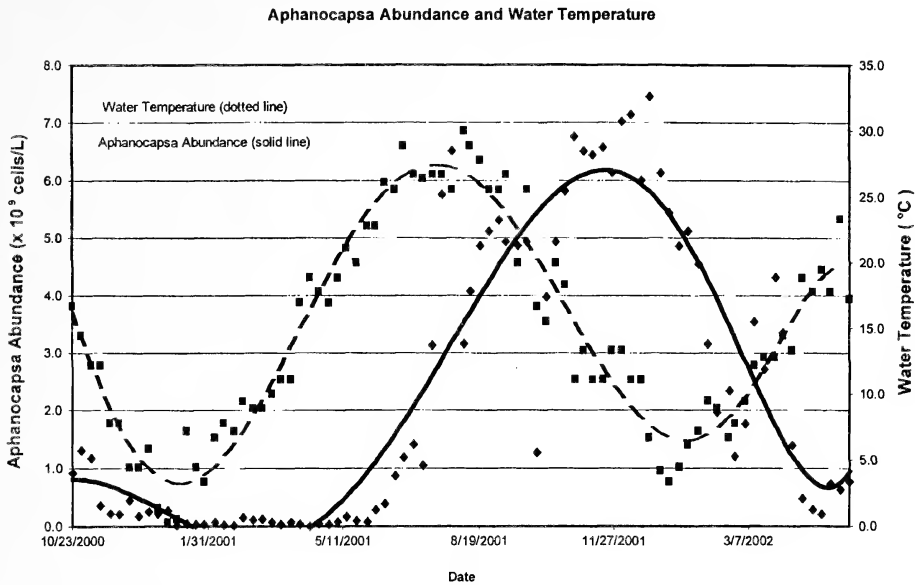


FIGURE 5. Comparison of *Aphanocapsa holsatica* and water temperature. Polynomial best fit lines are used for each data series.

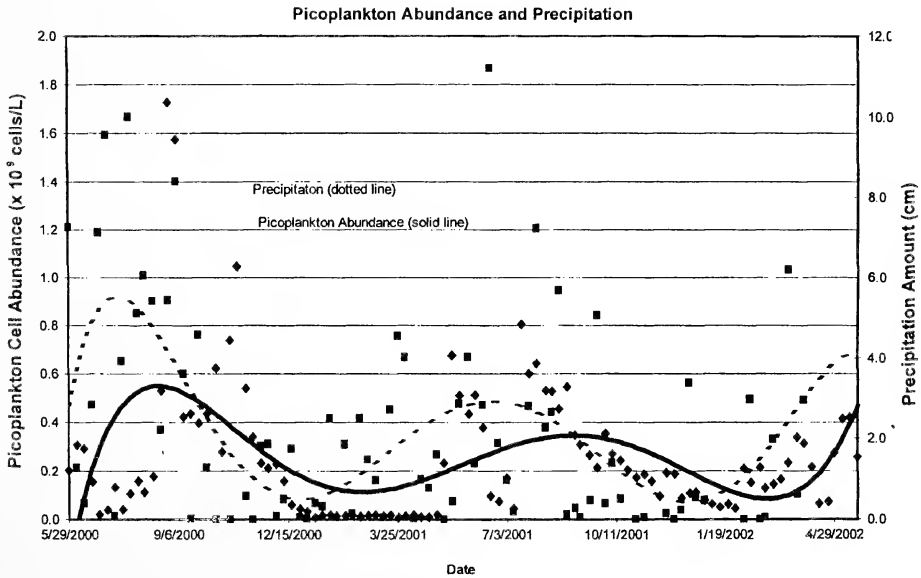


FIGURE 6. Comparison of picoplankton abundance and precipitation amount. Polynomial best fit lines are used for each data series.

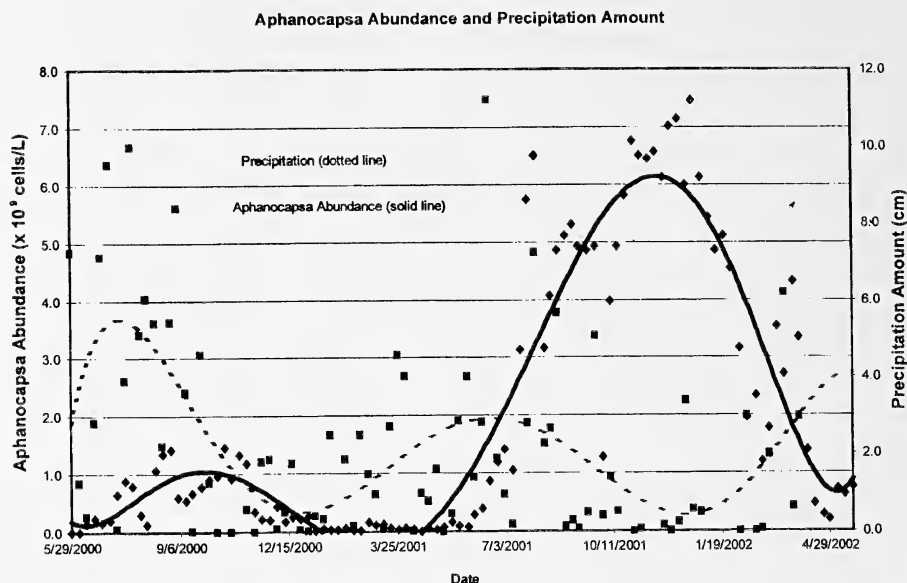


FIGURE 7. Comparison of *Aphanocapsa holsatica* and precipitation amounts. Polynomial best fit lines are used for each data series.

## DISCUSSION

The inverse relationship of *Aphanocapsa holsatica* and picoplankton abundance (Figure 3) suggests these two plankton groups are interrelated. In his review of autotrophic picoplankton, Marshall (2002) stated there are numerous freshwater colonial cyanobacteria such as *Aphanothece*, *Aphanocapsa*, *Merismopedia*, etc. with cells  $<2.0\ \mu\text{m}$  in size. Komárek (2000) describes species of *Aphanocapsa* with individual cells within the size range of picoplankton. Likewise, Komárek (2000) stated reproduction of *Aphanocapsa holsatica* involves the disintegration of colonies into solitary cells. With this information, it is likely for *Aphanocapsa holsatica*, during certain phases of its growth (i.e. reproduction) and perhaps under certain environmental conditions, to contribute to the picoplankton component.

It is widely understood that plankton are influenced by a variety of unique physical, chemical, and environmental factors. Data from this study indicated colonies of *Aphanocapsa holsatica* were being influenced by temperature and precipitation. When water temperatures were lower, *Aphanocapsa holsatica* abundance remained high (colonies remained intact) and picoplankton abundance was relatively low. During higher temperatures, *Aphanocapsa holsatica* was most likely undergoing increased cell division where colonies disaggregated and solitary cells of *Aphanocapsa holsatica* contributed to picoplankton abundance.

Over the first year of this study, rainfall amounts were over 1.5 times higher than the second year. *Aphanocapsa holsatica* populations were relatively low during the first year of the study when there was more rainfall and populations increased in number when rainfall decreased. The opposite was true for picoplankton abundance where a decrease in abundance was observed over the two year study. The fact that picoplankton abundance patterns mimicked precipitation amounts, suggests this freshwater input has a direct influence on *Aphanocapsa holsatica* colony disaggregation. There is

support for this suggestion from culture studies on other colonial cyanoprokaryotes by Parker (1982). She observed *Microcystis* sp. colonies dispersing into unicells when distilled water was added to the colonies. Furthermore, she suggested because of this occurrence *Microcystis* cells could be mistakenly identified in the field as a unicellular organism.

The degree to which this dispersion response of *Aphanocapsa holsatica* to freshwater input is evolutionary in nature, simply a chemical response (Parker, 1982), or some reproductive strategy to increase population abundance is difficult to discern. However, colony dispersion during storm water input would be beneficial to the survival of *Aphanocapsa holsatica*, and nutrient input during rain events is likely to increase the chance for species propagation. When picoplankton diversity is modified, microbial food web dynamics that include carbon trophodynamics and microzooplankton grazing rates are also expected to vary. These changes may result in significant shifts at higher trophic levels, thus influencing overall lake productivity and efficiency.

### CONCLUSION

Data from this study suggests a major contributor to picoplankton abundance in Lake Joyce is from the disaggregation of *Aphanocapsa holsatica* colonies. The cosmopolitan nature of *Aphanocapsa holsatica* infers this association may also occur in other aquatic habitats. To verify species specific relationships of picoplankton and *Aphanocapsa holsatica*, DNA techniques (Krienitz et al., 1999; Moon-van der Staay et al., 2000; Lopez-Garcia et al., 2001 and Moon-van-der Staay et al., 2001) would be required and should be the focus of further research. Two environmental factors appear to trigger the release of individual cells of *Aphanocapsa holsatica*; these included temperature and rainwater input. This study emphasizes the importance of long term, high frequency studies on time scales that more accurately correspond with picoplankton and *Aphanocapsa holsatica* growth patterns under changing environmental conditions.

### ACKNOWLEDGMENTS

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